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HYDROXY ACID INTEGRIN ANTAGONISTS

The present application claims priority under Title 35, United States Code, §119 of United States Provisional applications Serial No. 60/235,616 filed September 27, 2000 and Serial No. 60/60/241,656 filed October 10, 2000.

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Field of the Invention

The present invention relates to pharmaceutical agents which are $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrin antagonists and as such are useful in pharmaceutical compositions and in methods for treating conditions mediated by $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrins.

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Background of the Invention

Integrins are a group of cell surface glycoproteins which mediate cell adhesion and therefore are useful mediators of cell adhesion interactions which occur during various biological processes. Integrins are heterodimers composed of noncovalently linked α and β polypeptide subunits. Currently eleven different α subunits have been identified and six different β subunits have been identified. The various α subunits can combine with various β subunits to form distinct integrins.

The integrin identified as $\alpha_v\beta_3$ (also known as the vitronectin receptor) has been identified as an integrin which plays a role in various conditions or disease states including tumor metastasis, solid tumor growth (neoplasia), osteoporosis (Ross, *et al.*, *J. Biol. Chem.*, 1987, 262, 7703), Paget's disease, humoral hypercalcemia of malignancy (Carron *et al.*, *Cancer Res.* 1998, 58, 1930), osteopenia (Lark *et al.*, *J Bone Miner Res.* 2001, 16, 319), endometriosis (Healy *et al.*, *Hum. Reproductive Update*, 1998, 4, 736), angiogenesis, including tumor angiogenesis (Cheresh, *Cancer Metastasis Rev.*, 1991, 10, 3-10 and Brooks, *et al.*, *Cell*, 1994, 79, 1157), retinopathy including macular degeneration (Friedlander *et al.*, *Proc. Natl. Acad. Sci USA* 1996, 93, 9764), arthritis, including rheumatoid arthritis (Badger *et al.*, *Arthritis Rheum*, 2001, 44, 128), periodontal disease, psoriasis and smooth muscle cell migration (e.g. restenosis and

5 atherosclerosis, (Brown *et al.*, *Cardiovascular Res.*, 1994, 28, 1815). The compounds of the present invention are $\alpha_v\beta_3$ antagonists and can be used, alone or in combination with other therapeutic agents, in the treatment or modulation of various conditions or disease states described above. Additionally, it has been found that such agents would be useful as

10 antivirals, antifungals and antimicrobials. Thus, compounds which selectively antagonize $\alpha_v\beta_3$ would be beneficial for treating such conditions.

The integrin $\alpha_v\beta_5$ plays a role in neovascularization. Antagonists of the $\alpha_v\beta_5$ integrin will inhibit neovascularization and will be useful for treating and preventing angiogenesis metastasis, tumor growth, macular degeneration and diabetic retionopathy. M.C. Friedlander, *et al.*, *Science*, 270, 1500-1502 (1995) disclose that a monoclonal antibody for $\alpha_v\beta_5$ inhibits VEGF-induced angogenesis in the rabbit cornea and the chick chorioallantoic membrane model. Therefore, it would be useful to antagonize both the $\alpha_v\beta_5$ and the $\alpha_v\beta_3$ receptor. Such "mixed $\alpha_v\beta_5/\alpha_v\beta_3$ antagonists" or "dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists" would be useful for treating or preventing angiogenesis, tumor metastasis, tumor growth, diabetic retinopathy, macular degeneration, atherosclerosis and osteoporosis.

It has been shown that the $\alpha_v\beta_3$ integrin and other α_v containing integrins bind to a number of Arg-Gly-Asp (RGD) containing matrix macromolecules. Compounds containing the RGD sequence mimic extracellular matrix ligands so as to bind to cell surface receptors. However, it is also known that RGD peptides in general are non-selective for RGD dependent integrins. For example, most RGD peptides which bind to $\alpha_v\beta_3$ also bind to $\alpha_v\beta_5$, $\alpha_v\beta_1$ and $\alpha_{IIb}\beta_3$. Antagonism of platelet 30 $\alpha_{IIb}\beta_3$ (also known as the fibrinogen receptor) is known to block platelet aggregation in humans. In order to avoid bleeding side-effects when treating the conditions or disease states associated with the integrin $\alpha_v\beta_3$, it would be beneficial to develop compounds which are selective antagonists of $\alpha_v\beta_3$ as opposed to $\alpha_{IIb}\beta_3$.

35 Further, it has not been established in the art that sparing $\alpha_v\beta_6$ integrin would be a beneficial property to be incorporated in the design of

5 antagonists of $\alpha_v\beta_3$. Rather, $\alpha_v\beta_6$ has been identified as a target for
antagonists because it is highly expressed in many carcinoma cell lines, and
has been shown to enhance the proliferative capacity of a colon
carcinoma cell line both *in vivo* and *in vitro* (Agrez *et al.*, 1994, *J. Cell Biol.*
127, 547). Additionally, $\alpha_v\beta_6$ is expressed during the later stages of wound
10 healing and remains expressed until the wound is closed (See Christofidou-
Solomidou, *et al.*, 1997 *American J. of Pathol.*, 151, 975), and therefore it is
believed that $\alpha_v\beta_6$ plays a role in the remodeling of the vasculature during
the later stages of angiogenesis. Accordingly, antagonists of $\alpha_v\beta_6$ are seen
as useful in treating or preventing cancer by inhibiting tumor growth and
15 metastasis (see, for example, United States Patent 6,211,191).

Tumor cell invasion occurs by a three step process: 1) tumor cell
attachment to extracellular matrix; 2) proteolytic dissolution of the matrix;
and 3) movement of the cells through the dissolved barrier. This process
can occur repeatedly and can result in metastases at sites distant from the
20 original tumor.

Seftor *et al.* (*Proc. Natl. Acad. Sci. USA*, Vol. 89 (1992) 1557-1561)
have shown that the $\alpha_v\beta_3$ integrin has a biological function in melanoma
cell invasion. Montgomery *et al.*, (*Proc. Natl. Acad. Sci. USA*, Vol. 91
(1994) 8856-60) have demonstrated that the integrin $\alpha_v\beta_3$ expressed on
25 human melanoma cells promotes a survival signal, protecting the cells from
apoptosis. Mediation of the tumor cell metastatic pathway by interference
with the $\alpha_v\beta_3$ integrin cell adhesion receptor to impede tumor metastasis
would be beneficial.

Brooks *et al.* (*Cell*, Vol. 79 (1994) 1157-1164) have demonstrated
30 that antagonists of $\alpha_v\beta_3$ provide a therapeutic approach for the treatment of
neoplasia (inhibition of solid tumor growth) since systemic administration of
 $\alpha_v\beta_3$ antagonists causes dramatic regression of various histologically
distinct human tumors.

The adhesion receptor integrin $\alpha_v\beta_3$ was identified as a marker of
35 angiogenic blood vessels in chick and man and therefore such receptor
plays a critical role in angiogenesis or neovascularization. Angiogenesis is

5 characterized by the invasion, migration and proliferation of smooth muscle and endothelial cells. Antagonists of $\alpha_v\beta_3$ inhibit this process by selectively promoting apoptosis of cells in neovasculature. The growth of new blood vessels, or angiogenesis, also contributes to pathological conditions such as diabetic retinopathy including macular degeneration (Adamis *et al.*,
10 *Amer. J. Ophthalm.*, Vol. 118, (1994) 445-450) and rheumatoid arthritis (Peacock *et al.*, *J. Exp. Med.*, Vol. 175, (1992), 1135-1138). Therefore, $\alpha_v\beta_3$ antagonists would be useful therapeutic agents for treating such conditions associated with neovascularization (Brooks *et al.*, *Science*, Vol. 264, (1994), 569-571).

15 It has been reported that the cell surface receptor $\alpha_v\beta_3$ is the major integrin on osteoclasts responsible for attachment to bone. Osteoclasts cause bone resorption and when such bone resorbing activity exceeds bone forming activity it results in osteoporosis (loss of bone), which leads to an increased number of bone fractures, incapacitation and increased
20 mortality. Antagonists of $\alpha_v\beta_3$ have been shown to be potent inhibitors of osteoclastic activity both *in vitro* [Sato *et al.*, *J. Cell. Biol.*, Vol. 111 (1990) 1713-1723] and *in vivo* [Fisher *et al.*, *Endocrinology*, Vol. 132 (1993) 1411-1413]. Antagonism of $\alpha_v\beta_3$ leads to decreased bone resorption and therefore restores a normal balance of bone forming and resorbing activity.
25 Thus it would be beneficial to provide antagonists of osteoclast $\alpha_v\beta_3$ which are effective inhibitors of bone resorption and therefore are useful in the treatment or prevention of osteoporosis.

The role of the $\alpha_v\beta_3$ integrin in smooth muscle cell migration also makes it a therapeutic target for prevention or inhibition of neointimal
30 hyperplasia which is a leading cause of restenosis after vascular procedures (Choi *et al.*, *J. Vasc. Surg.* Vol. 19(1) (1994) 125-34). Prevention or inhibition of neointimal hyperplasia by pharmaceutical agents to prevent or inhibit restenosis would be beneficial.

White (*Current Biology*, Vol. 3(9)(1993) 596-599) has reported that
35 adenovirus uses $\alpha_v\beta_3$ for entering host cells. The integrin appears to be required for endocytosis of the virus particle and may be required for

5 penetration of the viral genome into the host cell cytoplasm. Thus compounds which inhibit $\alpha\beta_3$ would find usefulness as antiviral agents.

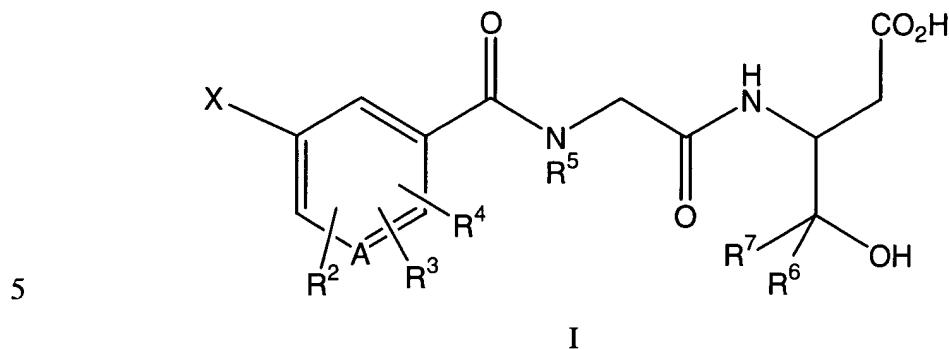
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Summary of the Invention

The compounds of this invention are 1) $\alpha_v\beta_3$ integrin antagonists; or 2) $\alpha_v\beta_5$ integrin antagonists; or 3) mixed or dual $\alpha_v\beta_3/\alpha_v\beta_5$ antagonists. The present invention includes compounds which inhibit the respective 10 integrins and also includes pharmaceutical compositions comprising such compounds. The present invention further provides for methods for treating or preventing conditions mediated by the $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ receptors in a mammal in need of such treatment comprising administering a therapeutically effective amount of the compounds of the present 15 invention and pharmaceutical compositions of the present invention. Administration of such compounds and compositions of the present invention inhibits angiogenesis, tumor metastasis, tumor growth, osteoporosis, Paget's disease, humoral hypercalcemia of malignancy, retinopathy, macular degeneration, arthritis, periodontal disease, smooth 20 muscle cell migration, including restenosis and atherosclerosis, and viral diseases.

The compounds of the present invention further show greater selectivity for the $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrin than for the $\alpha_v\beta_6$ integrin. It has been found that the selective antagonism of the $\alpha_v\beta_3$ integrin is desirable in 25 that the $\alpha_v\beta_6$ integrin may play a role in normal physiological processes of tissue repair and cellular turnover that routinely occur in the skin and pulmonary tissue, and the inhibition of this function can be deleterious. Therefore, compounds of the present invention which selectively inhibit the $\alpha_v\beta_3$ integrin as opposed to the $\alpha_v\beta_6$ integrin have reduced side-effects 30 associated with inhibition of the $\alpha_v\beta_6$ integrin.

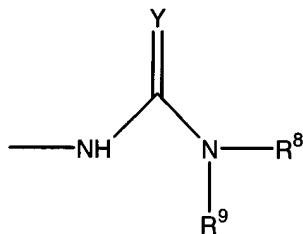
The present invention relates to a class of compounds represented by the Formula I.



or a pharmaceutically acceptable salts thereof wherein

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X is



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Y is selected from the group consisting of N-R¹, O, and S;

A is N or C;

20 R¹ is selected from the group consisting of H, alkyl, aryl, hydroxy, alkoxy, cyano, nitro, amino, alkenyl, alkynyl, amido, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxycarbonyl, alkylthiocarbonyl, arylthiocarbonyl, acyloxymethoxycarbonyl, alkyl optionally substituted with one or more substituent selected from lower alkyl, halogen,

25 hydroxyl, haloalkyl, cyano, nitro, carboxyl, amino, alkoxy, aryl or aryl optionally substituted with one or more halogen, haloalkyl, lower alkyl, alkoxy, cyano, alkylsulfonyl, alkylthio, nitro, carboxyl, amino, hydroxyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, or

5 fused monocyclic heterocycles, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, hydroxy, lower alkyl, alkoxy, methylenedioxy, ethylenedioxy, cyano, nitro, alkylthio, alkylsulfonyl, sulfonic acid, sulfonamide, carboxyl derivatives, amino, aryl, fused aryl, monocyclic heterocycles and fused monocyclic heterocycle, monocyclic
10 heterocycles, and monocyclic heterocycles optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy, amino, nitro, hydroxy, carboxyl derivatives, cyano, alkylthio, alkylsulfonyl, sulfonic acid, sulfonamide, aryl or fused aryl; or

15 R¹ taken together with R⁸ forms a 4-12 membered dinitrogen containing heterocycle optionally substituted with one or more substituent selected from the group consisting of lower alkyl, hydroxy, keto, alkoxy, halo, phenyl, amino, carboxyl or carboxyl ester, and fused phenyl; or

20 R¹ taken together with R⁸ forms a 5 membered heteroaromatic ring optionally substituted with one or more substituent selected from lower alkyl, phenyl and hydroxy; or

25 R¹ taken together with R⁸ forms a 5 membered heteroaromatic ring fused with a phenyl group;

R⁸ (when not taken together with R¹) and R⁹ are independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, aralkyl, amino, alkylamino, hydroxy, alkoxy, arylamino, amido, alkylcarbonyl, arylcarbonyl,
30 alkoxycarbonyl, aryloxy, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxy-carbonyl, alkylthiocarbonyl, arylthiocarbonyl, acyloxymethoxycarbonyl, cycloalkyl, bicycloalkyl, aryl, acyl, benzoyl, alkyl optionally substituted with one or more substituent selected from lower alkyl, halogen, hydroxy, haloalkyl, cyano, nitro, carboxyl derivatives, amino, alkoxy, thio, alkylthio,
35 sulfonyl, aryl, aralkyl, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy, methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano,

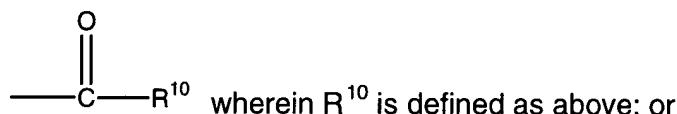
5 nitro, carboxyl derivatives, aryloxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethyl, sulfonyl, alkylsulfonyl, haloalkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, fused monocyclic heterocycles, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl,

10 alkoxy, methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, carboxyl derivatives, aryloxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethylsulfonyl, alkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, or fused monocyclic heterocycles, monocyclic heterocycles,

15 monocyclic heterocycles optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy, aryloxy, amino, nitro, hydroxy, carboxyl derivatives, cyano, alkylthio, alkylsulfonyl, aryl, fused aryl, monocyclic and bicyclic heterocyclicalkyls, $-\text{SO}_2\text{R}^{10}$ wherein R^{10} is selected from the group consisting of alkyl, aryl and monocyclic

20 heterocycles, all optionally substituted with one or more substituent selected from the group consisting of halogen, haloalkyl, alkyl, alkoxy, cyano, nitro, amino, acylamino, trifluoroalkyl, amido, alkylaminosulfonyl, alkylsulfonyl, alkylsulfonylamino, alkylamino, dialkylamino, trifluoromethylthio, trifluoroalkoxy, trifluoromethylsulfonyl, aryl, aryloxy, thio, alkylthio, and monocyclic heterocycles; and

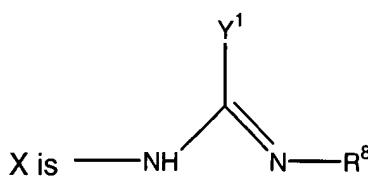
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NR⁸ and R⁹ taken together form a 4-12 membered mononitrogen containing monocyclic or bicyclic ring optionally substituted with one or more substituent selected from lower alkyl, carboxyl derivatives, aryl or hydroxy and wherein said ring optionally contains a heteroatom selected from the group consisting of O, N and S;

35 or

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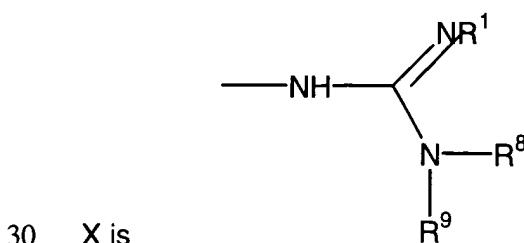


wherein Y¹ is selected from the group consisting of alkyl, cycloalkyl, bicycloalkyl, aryl, monocyclic heterocycles, alkyl optionally substituted with

- 10 aryl which can also be optionally substituted with one or more substituent selected from halogen, haloalkyl, alkyl, nitro, hydroxy, alkoxy, aryloxy, aryl, or fused aryl, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, hydroxy, alkoxy, aryloxy, aryl, fused aryl, nitro, methylenedioxy, ethylenedioxy, or alkyl, alkynyl, alkenyl, -S-R¹¹ and -
- 15 OR¹¹ wherein R¹¹ is selected from the group consisting of H, alkyl, aralkyl, aryl, alkenyl, and alkynyl, or R¹¹ taken together with R⁸ forms a 4-12 membered mononitrogen and monosulfur or monooxygen containing heterocyclic ring optionally substituted with lower alkyl, hydroxy, keto, phenyl, carboxyl or carboxyl ester, and fused phenyl, or R¹¹ taken together
- 20 with R⁸ is thiazole, oxazole, benzoxazole, or benzothiazole;

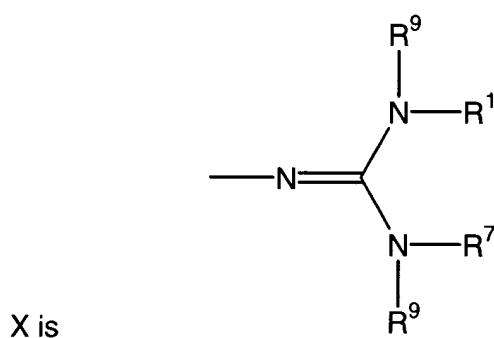
R⁸ is defined as above; or

- Y¹ (when Y¹ is carbon) taken together with R⁸ forms a 4-12 membered
- 25 mononitrogen or dinitrogen containing ring optionally substituted with alkyl, aryl, keto or hydroxy; or



5 wherein R¹ and R⁸ taken together form a 5-8 membered dinitrogen containing heterocycle optionally substituted with one or more substituent selected from the group consisting of lower alkyl, hydroxy, keto, phenyl, or carboxyl derivatives; and R⁹ is selected from the group consisting of alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxycarbonyl, alkylthiocarbonyl, arylthiocarbonyl, or acyloxymethoxycarbonyl; or

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15 wherein R¹ and R⁸ taken together form a 5-8 membered dinitrogen containing heterocycle optionally substituted with hydroxy, keto, phenyl, or alkyl; and

R⁹ are both selected from the group consisting of alkylcarbonyl,
20 arylcarbonyl, alkoxy carbonyl, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxycarbonyl, alkylthiocarbonyl, arylthiocarbonyl and acyloxymethoxycarbonyl;

R², R³ and R⁴ are independently selected from one or more substituent
25 selected from the group consisting of H, alkyl, hydroxy, alkoxy, aryloxy, halogen, haloalkyl, haloalkoxy, nitro, amino, alkylamino, acylamino, dialkylamino, cyano, alkylthio, alkylsulfonyl, carboxyl derivatives, trihaloacetamide, acetamide, aryl, fused aryl, cycloalkyl, thio, monocyclic heterocycles, fused monocyclic heterocycles, and X, wherein X is defined
30 as above;

5 R⁵, R⁶ and R⁷ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, carboxyl derivatives, haloalkyl, cycloalkyl, monocyclic heterocycles, monocyclic heterocycles optionally substituted with alkyl, halogen, haloalkyl, cyano, hydroxy, aryl, fused aryl, nitro, alkoxy, aryloxy, alkylsulfonyl, arylsulfonyl, sulfonamide, thio, alkylthio,

10 carboxyl derivatives, amino, amido, alkyl optionally substituted with one or more of halo, haloalkyl, hydroxy, alkoxy, aryloxy, thio, alkylthio, alkynyl, alkenyl, alkyl, arylthio, alkylsulfoxide, alkylsulfonyl, arylsulfoxide, arylsulfonyl, cyano, nitro, amino, alkylamino, dialkylamino, alkylsulfonamide, arylsulfonamide, acylamide, carboxyl derivatives, sulfonamide,

15 sulfonic acid, phosphonic acid derivatives, phosphinic acid derivatives, aryl, arylthio, arylsulfoxide, or arylsulfone all optionally substituted on the aryl ring with halo, alkyl, haloalkyl, cyano, nitro, hydroxy, carboxyl derivatives, alkoxy, aryloxy, amino, alkylamino, dialkylamino, amido, aryl, fused aryl, monocyclic heterocycles, and fused monocyclic heterocycles,

20 monocyclic heterocyclicthio, monocyclic heterocyclicsulfoxide, and monocyclic heterocyclic sulfone, which can be optionally substituted with halo, haloalkyl, nitro, hydroxy, alkoxy, fused aryl, or alkyl, alkylcarbonyl, haloalkylcarbonyl, and arylcarbonyl, aryl optionally substituted in one or more positions with halo, haloalkyl, alkyl, alkoxy, aryloxy, methylenedioxy,

25 ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, acyloxy, carboxyl derivatives, carboxyalkoxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethylsulfonyl, alkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles and fused monocyclic heterocycles.

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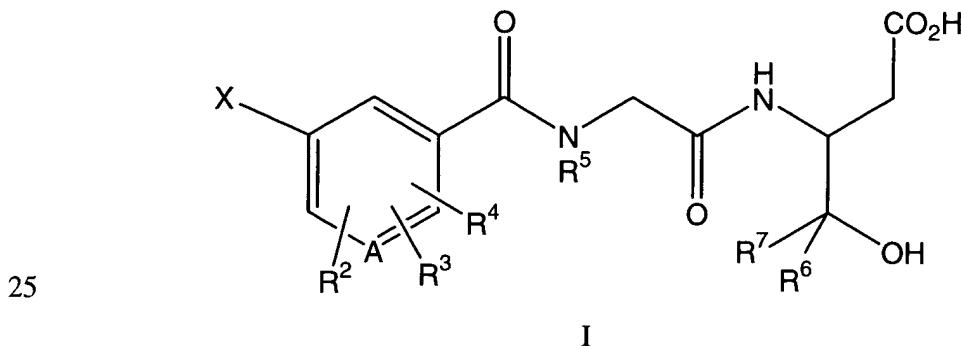
The compounds according to Formula I can exist in various isomers, enantiomers, tautomers, racemates and polymorphs, and all such forms are meant to be included.

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5 It is another object of the invention to provide pharmaceutical compositions comprising compounds of the Formula I. Such compounds and compositions are useful in selectively inhibiting or antagonizing the $\alpha_v \beta_3$ and/or $\alpha_v \beta_5$ integrins and therefore in another embodiment the present invention relates to a method of selectively inhibiting or
10 antagonizing the $\alpha_v \beta_3$ and/or $\alpha_v \beta_5$ integrin. The invention further involves treating or inhibiting pathological conditions associated therewith such as osteoporosis, humoral hypercalcemia of malignancy, Paget's disease, tumor metastasis, solid tumor growth (neoplasia), angiogenesis, including tumor angiogenesis, retinopathy including macular degeneration and
15 diabetic retinopathy, arthritis, including rheumatoid arthritis, periodontal disease, psoriasis, smooth muscle cell migration and restenosis in a mammal in need of such treatment. Additionally, such pharmaceutical agents are useful as antiviral agents, and antimicrobials.

20 Detailed Description

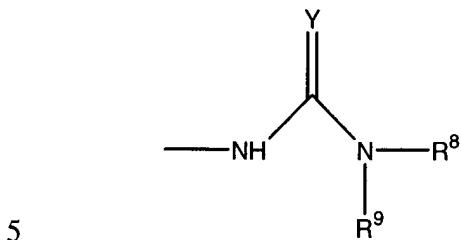
In its broadest sense, the invention relates to compounds represented by Formula I



or a pharmaceutically acceptable salts thereof wherein

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X is



Y is selected from the group consisting of N-R¹, O, and S;

10 A is N or C;

R¹ is selected from the group consisting of H, alkyl, aryl, hydroxy, alkoxy, cyano, nitro, amino, alkenyl, alkynyl, amido, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxycarbonyl,

15 alkylthiocarbonyl, arylthiocarbonyl, acyloxymethoxycarbonyl, alkyl optionally substituted with one or more substituent selected from lower alkyl, halogen, hydroxyl, haloalkyl, cyano, nitro, carboxyl, amino, alkoxy, aryl or aryl optionally substituted with one or more halogen, haloalkyl, lower alkyl, alkoxy, cyano, alkylsulfonyl, alkylthio, nitro, carboxyl, amino, hydroxyl,
20 sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, or fused monocyclic heterocycles, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, hydroxy, lower alkyl, alkoxy, methylenedioxy, ethylenedioxy, cyano, nitro, alkylthio, alkylsulfonyl, sulfonic acid, sulfonamide, carboxyl derivatives, amino, aryl, fused aryl,
25 monocyclic heterocycles and fused monocyclic heterocycle, monocyclic heterocycles, and monocyclic heterocycles optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy, amino, nitro, hydroxy, carboxyl derivatives, cyano, alkylthio, alkylsulfonyl, sulfonic acid, sulfonamide, aryl or fused aryl; or

30

R¹ taken together with R⁸ forms a 4-12 membered dinitrogen containing heterocycle optionally substituted with one or more substituent selected

5 from the group consisting of lower alkyl, hydroxy, keto, alkoxy, halo, phenyl, amino, carboxyl or carboxyl ester, and fused phenyl; or

10 R¹ taken together with R⁸ forms a 5 membered heteroaromatic ring optionally substituted with one or more substituent selected from lower alkyl, phenyl and hydroxy; or

15 R¹ taken together with R⁸ forms a 5 membered heteroaromatic ring fused with a phenyl group;

20 R⁸ (when not taken together with R¹) and R⁹ are independently selected from the group consisting of H, alkyl, alkenyl, alkynyl, aralkyl, amino, alkylamino, hydroxy, alkoxy, arylamino, amido, alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aryloxy, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxy carbonyl, alkylthiocarbonyl, arylthiocarbonyl, acyloxymethoxycarbonyl,

25 cycloalkyl, bicycloalkyl, aryl, acyl, benzoyl, alkyl optionally substituted with one or more substituent selected from lower alkyl, halogen, hydroxy, haloalkyl, cyano, nitro, carboxyl derivatives, amino, alkoxy, thio, alkylthio, sulfonyl, aryl, aralkyl, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy,

30 methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, carboxyl derivatives, aryloxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethyl, sulfonyl, alkylsulfonyl, haloalkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, fused monocyclic heterocycles, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy, methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, carboxyl derivatives, aryloxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethylsulfonyl, alkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, or fused monocyclic heterocycles, monocyclic heterocycles, monocyclic heterocycles optionally substituted with one or more

5 substituent selected from halogen, haloalkyl, lower alkyl, alkoxy, aryloxy, amino, nitro, hydroxy, carboxyl derivatives, cyano, alkylthio, alkylsulfonyl, aryl, fused aryl, monocyclic and bicyclic heterocyclicalkyls, $-\text{SO}_2\text{R}^{10}$ wherein R^{10} is selected from the group consisting of alkyl, aryl and monocyclic heterocycles, all optionally substituted with one or more substituent

10 selected from the group consisting of halogen, haloalkyl, alkyl, alkoxy, cyano, nitro, amino, acylamino, trifluoroalkyl, amido, alkylaminosulfonyl, alkylsulfonyl, alkylsulfonylamino, alkylamino, dialkylamino, trifluoromethylthio, trifluoroalkoxy, trifluoromethylsulfonyl, aryl, aryloxy, thio, alkylthio, and monocyclic heterocycles; and

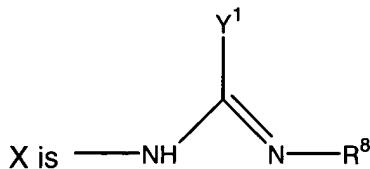


or NR^8 and R^9 taken together form a 4-12 membered mononitrogen containing monocyclic or bicyclic ring optionally substituted with one or more substituent selected from lower alkyl, carboxyl derivatives, aryl or hydroxy and wherein said ring optionally contains a heteroatom selected from the group consisting of O, N and S;

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or

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wherein Y' is selected from the group consisting of alkyl, cycloalkyl, bicycloalkyl, aryl, monocyclic heterocycles, alkyl optionally substituted with aryl which can also be optionally substituted with one or more substituent selected from halogen, haloalkyl, alkyl, nitro, hydroxy, alkoxy, aryloxy, aryl, or fused aryl, aryl optionally substituted with one or more substituent selected from halo, haloalkyl, hydroxy, alkoxy, aryloxy, aryl, fused aryl,

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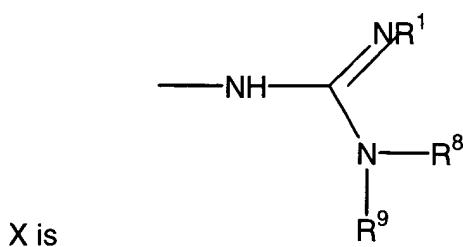
5 nitro, methylenedioxy, ethylenedioxy, or alkyl, alkynyl, alkenyl, -S-R¹¹ and -OR¹¹ wherein R¹¹ is selected from the group consisting of H, alkyl, aralkyl, aryl, alkenyl, and alkynyl, or R¹¹ taken together with R⁸ forms a 4-12 membered mononitrogen and monosulfur or monooxygen containing heterocyclic ring optionally substituted with lower alkyl, hydroxy, keto, phenyl, carboxyl or carboxyl ester, and fused phenyl, or R¹¹ taken together with R⁸ is thiazole, oxazole, benzoxazole, or benzothiazole;

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R⁸ is defined as above; or

15 Y¹ (when Y¹ is carbon) taken together with R⁸ forms a 4-12 membered mononitrogen or dinitrogen containing ring optionally substituted with alkyl, aryl, keto or hydroxy; or

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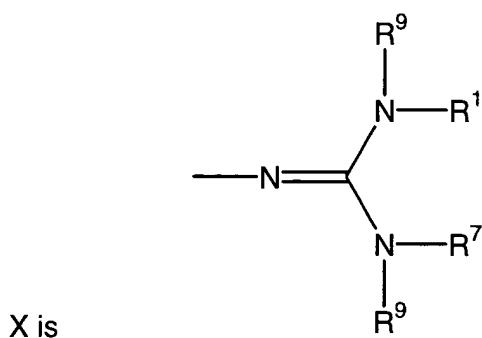


X is

wherein R¹ and R⁸ taken together form a 5-8 membered dinitrogen containing heterocycle optionally substituted with one or more substituent selected from the group consisting of lower alkyl, hydroxy, keto, phenyl, or carboxyl derivatives; and R⁹ is selected from the group consisting of alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aryloxycarbonyl, haloalkyl-carbonyl, haloalkoxycarbonyl, alkylthiocarbonyl, arylthiocarbonyl, or acyloxymethoxycarbonyl; or

25

30



wherein R¹ and R⁸ taken together form a 5-8 membered dinitrogen containing heterocycle optionally substituted with hydroxy, keto, phenyl, or 10 alkyl; and R⁹ are both selected from the group consisting of alkylcarbonyl, arylcarbonyl, alkoxy carbonyl, aryloxycarbonyl, haloalkylcarbonyl, haloalkoxycarbonyl, alkylthiocarbonyl, arylthiocarbonyl and acyloxy-methoxycarbonyl;

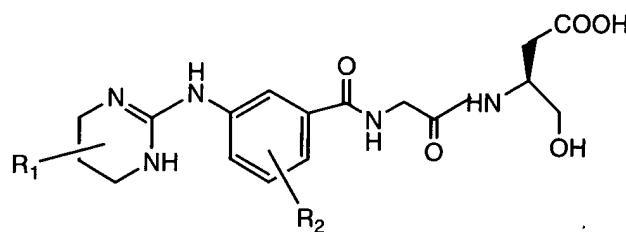
15 R², R³ and R⁴ are independently selected from one or more substituent selected from the group consisting of H, alkyl, hydroxy, alkoxy, aryloxy, halogen, haloalkyl, haloalkoxy, nitro, amino, alkylamino, acylamino, dialkylamino, cyano, alkylthio, alkylsulfonyl, carboxyl derivatives, trihaloacetamide, acetamide, aryl, fused aryl, cycloalkyl, thio, monocyclic 20 heterocycles, fused monocyclic heterocycles, and X, wherein X is defined above;

R⁵, R⁶ and R⁷ are independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, aryl, carboxyl derivatives, haloalkyl, 25 cycloalkyl, monocyclic heterocycles, monocyclic heterocycles optionally substituted with alkyl, halogen, haloalkyl, cyano, hydroxy, aryl, fused aryl, nitro, alkoxy, aryloxy, alkylsulfonyl, arylsulfonyl, sulfonamide, thio, alkylthio, carboxyl derivatives, amino, amido, alkyl optionally substituted with one or more of halo, haloalkyl, hydroxy, alkoxy, aryloxy, thio, alkylthio, alkynyl, 30 alkenyl, alkyl, arylthio,

5 alkylsulfoxide, alkylsulfonyl, arylsulfoxide, arylsulfonyl, cyano, nitro, amino, alkylamino, dialkylamino, alkylsulfonamide, arylsulfonamide, acylamide, carboxyl derivatives, sulfonamide, sulfonic acid, phosphonic acid derivatives, phosphinic acid derivatives, aryl, arylthio, arylsulfoxide, or arylsulfone all optionally substituted on the aryl ring with halo, alkyl, 10 haloalkyl, cyano, nitro, hydroxy, carboxyl derivatives, alkoxy, aryloxy, amino, alkylamino, dialkylamino, amido, aryl, fused aryl, monocyclic heterocycles, and fused monocyclic heterocycles, monocyclic heterocyclithio, monocyclic heterocyclicsulfoxide, and monocyclic heterocyclic sulfone, which can be optionally substituted with halo, 15 haloalkyl, nitro, hydroxy, alkoxy, fused aryl, or alkyl, alkylcarbonyl, haloalkylcarbonyl, and arylcarbonyl, aryl optionally substituted in one or more positions with halo, haloalkyl, alkyl, alkoxy, aryloxy, methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, acyloxy, carboxyl derivatives, carboxyalkoxy, amido, acylamino, amino, alkylamino, 20 dialkylamino, trifluoroalkoxy, trifluoromethylsulfonyl, alkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles and fused monocyclic heterocycles.

In another embodiment, the invention is represented by Formula II

25



II

or a pharmaceutically acceptable salt thereof, wherein R₁ and R₂ are selected from a group consisting of hydrogen, hydroxy alkyl haloalkyl and halo.

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5 The invention further relates to pharmaceutical compositions containing therapeutically effective amounts of the compounds of Formula I or II.

The invention also relates to a method of selectively inhibiting or antagonizing the $\alpha_v \beta_3$ integrin and/or the $\alpha_v \beta_5$ integrin and more

10 specifically relates to a method of inhibiting bone resorption, periodontal disease, osteoporosis, humoral hypercalcemia of malignancy, Paget's disease, tumor metastasis, solid tumor growth (neoplasia), angiogenesis, including tumor angiogenesis, retinopathy including macular degeneration and diabetic retinopathy, arthritis, including rheumatoid arthritis, smooth

15 muscle cell migration and restenosis by administering a therapeutically effective amount of a compound of the Formula I to achieve such inhibition together with a pharmaceutically acceptable carrier. More specifically it has been found that it advantageous to administer compounds which are $\alpha_v \beta_3$ and/or $\alpha_v \beta_5$ antagonists which compounds selectively inhibit the $\alpha_v \beta_3$ and/or

20 $\alpha_v \beta_5$ integrin as opposed to the $\alpha_v \beta_6$ integrin. It has now been found that such selectivity is beneficial in reducing unwanted side-effects.

To evaluate the selectivity of compounds between the integrins $\alpha_v \beta_3$ and $\alpha_v \beta_6$, cell-based assays are established using the 293 human embryonic kidney cell line as described herein. The compounds disclosed

25 herein have shown significant selectivity between the integrins $\alpha_v \beta_3$ and $\alpha_v \beta_6$. The selective antagonism of the $\alpha_v \beta_3$ integrin is viewed as desirable in this class of compounds, as $\alpha_v \beta_6$ may also play a role in normal physiological processes of tissue repair and cellular turnover that routinely occur in the skin and pulmonary tissues.

30

As used herein, the terms "alkyl" or "lower alkyl" refer to a straight chain or branched chain hydrocarbon radicals having from about 1 to about 10 carbon atoms, and more preferably 1 to about 6 carbon atoms. Examples of such alkyl radicals are methyl, ethyl, n-propyl, isopropyl, n-

35 butyl, isobutyl, sec-butyl, t-butyl, pentyl, neopentyl, hexyl, isohexyl, and the like.

5 As used herein the terms "alkenyl" or "lower alkenyl" refer to unsaturated acyclic hydrocarbon radicals containing at least one double bond and 2 to about 6 carbon atoms, which carbon-carbon double bond may have either cis or trans geometry within the alkenyl moiety, relative to groups substituted on the double bond carbons. Examples of such groups
10 are ethenyl, propenyl, butenyl, isobut enyl, pentenyl, hexenyl and the like.

As used herein the terms "alkynyl" or "lower alkynyl" refer to acyclic hydrocarbon radicals containing one or more triple bonds and 2 to about 6 carbon atoms. Examples of such groups are ethynyl, propynyl, butynyl, pentynyl, hexynyl and the like.

15 The term "cycloalkyl" as used herein means saturated or partially unsaturated cyclic carbon radicals containing 3 to about 8 carbon atoms and more preferably 4 to about 6 carbon atoms. Examples of such cycloalkyl radicals include cyclopropyl, cyclopropenyl, cyclobutyl, cyclopentyl, cyclohexyl, 2-cyclohexen-1-yl, and the like.

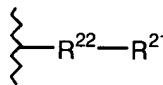
20 The term "aryl" as used herein denotes aromatic ring systems composed of one or more aromatic rings. Preferred aryl groups are those consisting of one, two or three aromatic rings. The term embraces aromatic radicals such as phenyl, pyridyl, naphthyl, thiophene, furan, biphenyl and the like.

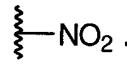
25 As used herein, the term "cyano" is represented by a radical $\text{--}\ddot{\text{C}}\text{N}$.
The terms "hydroxy" and "hydroxyl" as used herein are synonymous and are represented by a radical $\text{--}\ddot{\text{O}}\text{H}$.

30 The term "lower alkylene" or "alkylene" as used herein refers to divalent linear or branched saturated hydrocarbon radicals of 1 to about 6 carbon atoms.

As used herein the term "alkoxy" refers to straight or branched chain oxy containing radicals. Examples of alkoxy groups encompassed include methoxy, ethoxy, n-propoxy, n-butoxy, isopropoxy, isobutoxy, sec-butoxy, t-butoxy and the like.

35 As used herein the terms "arylalkyl" or "aralkyl" refer to a radical of

5 the formula  1 wherein R²¹ is aryl as defined above and R²² is an alkylene as defined above. Examples of aralkyl groups include benzyl, pyridylmethyl, naphthylpropyl, phenethyl and the like.

As used herein the term "nitro" is represented by a radical .

As used herein the term "halo" or "halogen" refers to bromo, chloro, fluoro or iodo.

As used herein the term "haloalkyl" refers to alkyl groups as defined above substituted with one or more of the same or different halo groups at one or more carbon atom. Examples of haloalkyl groups include trifluoromethyl, dichloroethyl, fluoropropyl and the like.

15 As used herein the term "carboxyl" or "carboxy" refers to a radical of the formula -COOH.

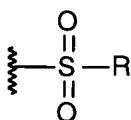
As used herein the term "carboxyl ester" refers to a radical of the formula -COOR²³ wherein R²³ is selected from the group consisting of H, alkyl, aralkyl or aryl as defined above.

20 As used herein the term "carboxyl derivative" refers to a radical of


the formula —C—Y⁷R²³ 2 wherein Y⁶ and Y⁷ are independently selected from the group consisting of O, N or S and R²³ is selected from the group consisting of H, alkyl, aralkyl and aryl as defined above.

As used herein the term "amino" is represented by a radical of the
25 formula -NH₂.

As used herein the term "alkylsulfonyl" or "alkylsulfone" refers to a

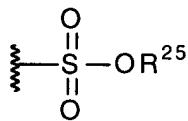
radical of the  where R²⁴ is alkyl as defined above.

30 As used herein the term "alkylthio" refers to a radical of the formula -SR²⁴ where R²⁴ is alkyl as defined above.

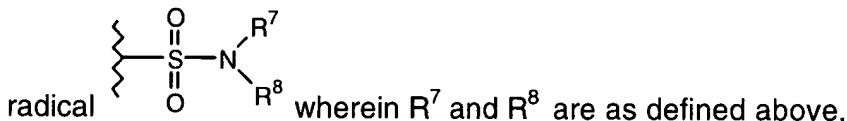
As used herein the term "sulfonic acid" refers to a

5

radical of the

wherein R²⁵ is alkyl as defined above.

As used herein the term "sulfonamide" or "sulfonamido" refers to a



10

As used herein the term "fused aryl" refers to an aromatic ring such as the aryl groups defined above fused to one or more phenyl rings.

Embraced by the term "fused aryl" is the radical naphthyl and the like.

As used herein the terms "monocyclic heterocycle" or "monocyclic heterocyclic" refer to a monocyclic ring containing from 4 to about 12

15

atoms, and more preferably from 5 to about 10 atoms, wherein 1 to 3 of the atoms are heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur with the understanding that if two or more different heteroatoms are present at least one of the heteroatoms must be nitrogen. Representative of such monocyclic heterocycles are imidazole, furan,

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pyridine, oxazole, pyran, triazole, thiophene, pyrazole, thiazole, thiadiazole, and the like.

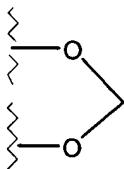
As used herein the term "fused monocyclic heterocycle" refers to a monocyclic heterocycle as defined above with a benzene fused thereto.

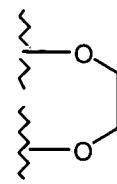
Examples of such fused monocyclic heterocycles include benzofuran,

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benzopyran, benzodioxole, benzothiazole, benzothiophene, benzimidazole and the like.

As used herein the term "methylenedioxy" refers to the radical



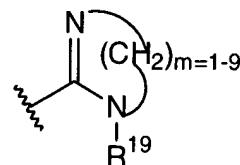


5 and the term "ethylenedioxy" refers to the radical

As used herein the term "4-12 membered dinitrogen containing

heterocycle refers to a radical of the formula

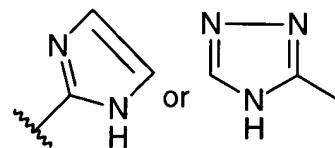
10



wherein m is 1 or 2 and R^{19} is H, alkyl, aryl, or aralkyl and more preferably refers to 4-9 membered ring and includes rings such as imidazoline.

As used herein the term "5-membered optionally substituted

15 heteroaromatic ring" includes for example a radical of the formula



and "5-membered heteroaromatic ring fused with a phenyl" refers to such a "5-membered heteroaromatic ring" with a phenyl fused thereto.

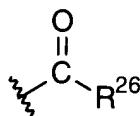
Representative of such 5-membered heteroaromatic rings fused with a

20 phenyl is benzimidazole.

As used herein the term "bicycloalkyl" refers to a bicyclic hydrocarbon radical containing 6 to about 12 carbon atoms which is saturated or partially unsaturated.

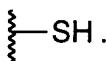
As used herein the term "acyl" refers to a radical of the formula

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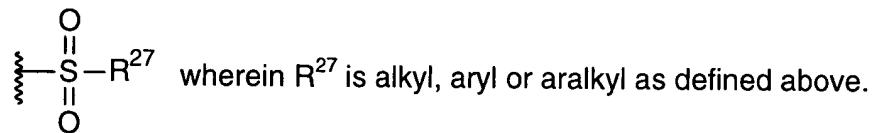


3 wherein R^{26} is alkyl, alkenyl, alkynyl, aryl or aralkyl and optionally substituted thereon as defined above. Encompassed by such radical are the groups acetyl, benzoyl and the like.

As used herein the term "thio" refers to a radical of the formula



5 As used herein the term "sulfonyl" refers to a radical of the formula



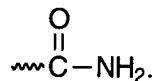
10 As used herein the term "haloalkylthio" refers to a radical of the formula -S-R²⁸ wherein R²⁸ is haloalkyl as defined above.

As used herein the term "aryloxy" refers to a radical of the formula
 $\begin{array}{c} \text{O} \\ \parallel \\ \text{C}-\text{R}^{29} \end{array}$ wherein R²⁹ is aryl as defined above.

As used herein the term "acylamino" refers to a radical of the

15 formula $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}^{30}-\text{C}-\text{NH}- \end{array}$ wherein R³⁰ is alkyl, aralkyl or aryl as defined above.

As used herein the term "amido" refers to a radical of the formula



20 As used herein the term "alkylamino" refers to a radical of the formula -NHR³² wherein R³² is alkyl as defined above.

As used herein the term "dialkylamino" refers to a radical of the formula -NR³³R³⁴ wherein R³³ and R³⁴ are the same or different alkyl groups as defined above.

25 As used herein the term "trifluoromethyl" refers to a radical of the formula $\begin{array}{c} \text{C F}_3. \end{array}$

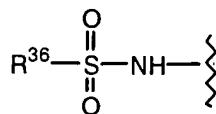
As used herein the term "trifluoroalkoxy" refers to a radical of the formula $\begin{array}{c} \text{F}_3\text{C}-\text{R}^{35}-\text{O}- \end{array}$ wherein R³⁵ is a bond or an alkylene as defined above.

As used herein the term "alkylaminosulfonyl" or "aminosulfonyl"

30 refers to a radical of the formula $\begin{array}{c} \text{R}^{36}-\text{N}-\text{H}-\text{S}-\text{O}- \end{array}$ wherein R³⁶ is alkyl as defined above.

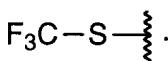
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As used herein the term "alkylsulfonylamino" or "alkylsulfonamide"

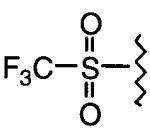
refers to a radical of the formula  wherein R³⁶ is alkyl as defined above.

10

As used herein the term "trifluoromethylthio" refers to a radical of the

formula .

As used herein the term "trifluoromethylsulfonyl" refers to a radical

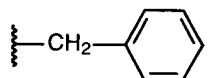
of the formula .

15

As used herein the term "4-12 membered mono-nitrogen containing monocyclic or bicyclic ring" refers to a saturated or partially unsaturated monocyclic or bicyclic ring of 4-12 atoms and more preferably a ring of 4-9 atoms wherein one atom is nitrogen. Such rings may optionally contain

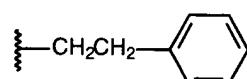
20 additional heteroatoms selected from nitrogen, oxygen or sulfur. Included within this group are morpholine, piperidine, piperazine, thiomorpholine, pyrrolidine, proline, azacycloheptene and the like.

As used herein the term "benzyl" refers to the radical



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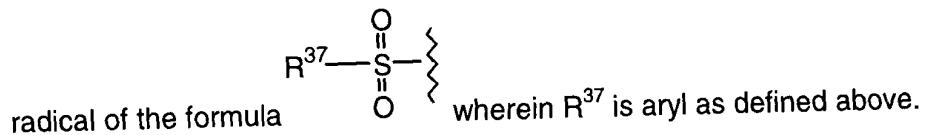
As used herein the term "phenethyl" refers to the radical



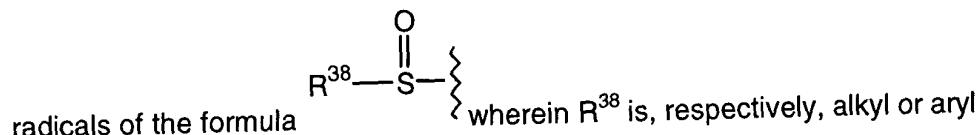
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As used herein the term "4-12 membered mono-nitrogen containing monosulfur or monooxygen containing heterocyclic ring" refers to a ring consisting of 4 to 12 atoms and more preferably 4 to 9 atoms wherein at least one atom is a nitrogen and at least one atom is oxygen or sulfur. Encompassed within this definition are rings such as thiazoline and the like.

5 As used herein the term "arylsulfonyl" or "arylsulfone" refers to a radical of the formula

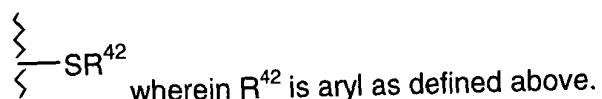


As used herein the terms "alkylsulfoxide" or "arylsulfoxide" refer to

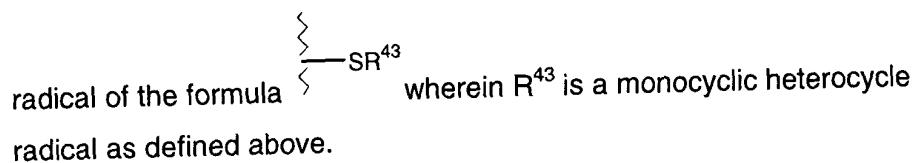


10 as defined above.

As used herein the term "arylthio" refers to a radical of the formula

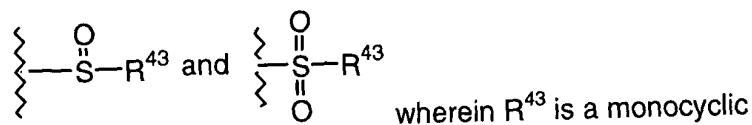


As used herein the term "monocyclic heterocycle thio" refers to a radical of the formula



15 radical as defined above.

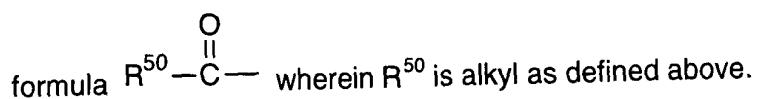
As used herein the terms "monocyclic heterocycle sulfoxide" and "monocyclic heterocycle sulfone" refer, respectively, to radicals



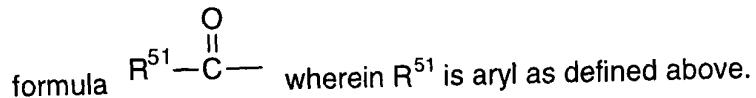
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heterocycle radical as defined above.

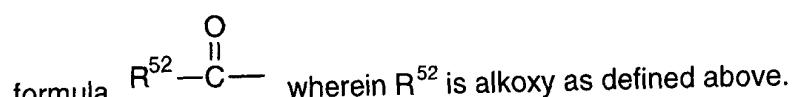
As used herein the term "alkylcarbonyl" refers to a radical of the



5 As used herein the term "arylcarbonyl" refers to a radical of the



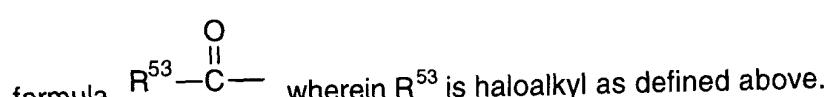
As used herein the term "alkoxycarbonyl" refers to a radical of the



As used herein the term "aryloxycarbonyl" refers to a radical of the



As used herein the term "haloalkoxycarbonyl" refers to a radical of the



As used herein the term "haloalkoxycarbonyl" refers to a radical of



15 As used herein the term "alkylthiocarbonyl" refers to a radical of the



As used herein the term "arylthiocarbonyl" refers to a radical of the



As used herein the term "acyloxymethoxycarbonyl" refers to a radical

20 of the formula $\text{R}^{54}-\overset{\text{O}}{\underset{\parallel}{\text{O}}}-\text{CH}_2-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-$ wherein R^{54} is acyl as defined
above.

As used herein the term "aryl amino" refers to a radical of the formula
 $\text{R}^{51}-\text{NH}-$ wherein R^{51} is aryl as defined above.

As used herein the term "acyloxy" refers to a radical of the formula
25 $\text{R}^{55}-\text{O}-$ wherein R^{55} is acyl as defined above.

5 As used herein the term "alkenylalkyl" refers to a radical of the formula $R^{50}-R^{57}$ —wherein R^{50} is an alkenyl as defined above and R^{57} is alkylene as defined above.

As used herein the term "alkenylene" refers to a linear hydrocarbon radical of 1 to about 8 carbon atoms containing at least one double bond.

10 As used herein the term "alkoxyalkyl" refers to a radical of the formula $R^{56}-R^{57}-$ wherein R^{56} is alkoxy as defined above and R^{57} is alkylene as defined above.

As used herein the term "alkynylalkyl" refers to a radical of the formula $R^{59}-R^{60}$ —wherein R^{59} is alkynyl as defined as above and R^{60} is alkylene as defined as above.

15 As used herein the term "alkynylene" refers to divalent alkynyl radicals of 1 to about 6 carbon atoms.

As used herein the term "allyl" refers of a radical of the formula $--CH_2CH=CH_2$.

20 As used herein the term "aminoalkyl" refers to a radical of the formula H_2N-R^{61} wherein R^{61} is alkylene as defined above.

As used herein the term "benzoyl" refers to the aryl radical C_6H_5-CO-

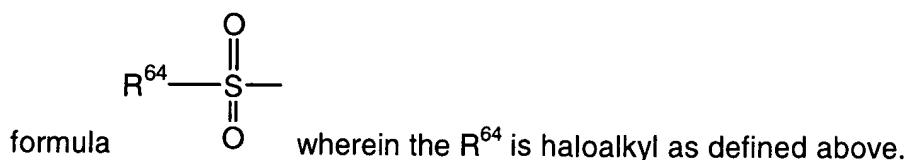
As used herein the term "carboxamide" or "carboxamido" refer to a radical of the formula $-CO-NH_2$.

25 As used herein the term "carboxyalkyl" refers to a radical $HOOC--R^{62}$ —wherein R^{62} is alkylene as defined as above.

As used herein the term "carboxylic acid" refers to the radical $-COOH$.

As used herein the term "ether" refers to a radical of the formula $R^{63}-O-$ wherein R^{63} is selected from the group consisting of alkyl, aryl and heteroaryl.

30 As used herein the term "haloalkylsulfonyl" refers to a radical of the formula $R^{64}-S(=O)-O-$ wherein the R^{64} is haloalkyl as defined above.



5 As used herein the term "heteroaryl" refers to an aryl radical contain at least one heteroatom.

As used herein the term "hydroxyalkyl" refers to a radical of the formula HO—R⁶⁵— wherein R⁶⁵ is alkylene as defined above.

10 As used herein the term "keto" refers to a carbonyl group joined to 2 carbon atoms.

As used herein the term "lactone" refers to an anhydro cyclic ester produced by intramolecular condensation of a hydroxy acid with the elimination of water.

15 As used herein the term "olefin" refers to an unsaturated hydrocarbon radical of the type C_nH_{2n}.

As used herein the term "sulfone" refers to a radical of the formula R⁶⁶—SO₂—.

As used herein the term "thioalkyl" refers to a radical of the formula R⁷⁷—S— wherein R⁷⁷ is alkyl as defined above.

20 As used herein the term "thioether" refers to a radical of the formula R⁷⁸—S— wherein R⁷⁸ is alkyl, aryl or heteroaryl.

As used herein the term "trifluoroalkyl" refers to an alkyl radical as defined above substituted with three halo radicals as defined above.

25 The term "composition" as used herein means a product which results from the mixing or combining of more than one element or ingredient.

The term "pharmaceutically acceptable carrier", as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

30 The term "therapeutically effective amount" shall mean that amount of drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system or animal that is being sought by a researcher or clinician.

35 The following is a list of abbreviations and the corresponding meanings as used interchangeably herein:

5	$^1\text{H-NMR}$ = proton nuclear magnetic resonance AcOH = acetic acid BOC = <u>tert</u> -butoxycarbonyl BuLi = butyl lithium Cat. = catalytic amount
10	CH_2Cl_2 = dichloromethane CH_3CN = acetonitrile CH_3I = iodomethane CHN analysis = carbon/hydrogen/nitrogen elemental analysis CHNCl analysis = carbon/hydrogen/nitrogen/chlorine elemental analysis
15	CHNS analysis = carbon/hydrogen/nitrogen/sulfur elemental analysis DEAD = diethylazodicarboxylate DIAD = diisopropylazodicarboxylate
20	DI water = deionized water DMA = <u>N,N</u> -dimethylacetamide DMAC = N,N-dimethylacetamide DMF = <u>N,N</u> -dimethylformamide
25	EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride Et = ethyl Et ₂ O = diethyl ether Et ₃ N = triethylamine EtOAc = ethyl acetate
30	EtOH = ethanol FAB MS = fast atom bombardment mass spectroscopy g = gram(s) HOBT = 1-hydroxybenzotriazole hydrate HPLC = high performance liquid chromatography
35	i-Pr = iso propyl i-Prop = iso propyl K_2CO_3 = potassium carbonate KMnO_4 = potassium permanganate KOH = potassium hydroxide
40	KSCN = potassium thiocyanate L = Liter LiOH = lithium hydroxide Me = methyl MeOH = methanol
45	mg = milligram MgSO_4 = magnesium sulfate ml = milliliter mL = milliliter MS = mass spectroscopy
50	NaH - sodium hydride NaHCO_3 = sodium bicarbonate NaOH = sodium hydroxide NaOMe = sodium methoxide $\text{NH}_4^+\text{HCO}_2^-$ = ammonium formate

5 NMR = nuclear magnetic resonance
Pd = palladium
Pd/C = palladium on carbon
Ph = phenyl
Pt = platinum
10 Pt/C = platinum on carbon
RPHPLC = reverse phase high performance liquid chromatography
RT = room temperature
t-BOC = tert-butoxycarbonyl
15 TFA = trifluoroacetic acid
THF = tetrahydrofuran
TLC - thin layer chromatography
TMS = trimethylsilyl
Δ = heating the reaction mixture
20 The compounds as shown above can exist in various isomeric forms and all such isomeric forms are meant to be included. Tautomeric forms are also included as well as pharmaceutically acceptable salts of such isomers and tautomers.
25 In the structures and formulas herein, a bond drawn across a bond of a ring can be to any available atom on the ring.
The term "pharmaceutically acceptable salt" refers to a salt prepared by contacting a compound of Formula I or II with an acid whose anion is generally considered suitable for human consumption. For use in
30 medicine, the salts of the compounds of this invention are non-toxic "pharmaceutically acceptable salts." Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention which are generally prepared by reacting the free base with a suitable organic or inorganic acid. Representative salts
35 include the following: acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride,
40 hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium

5 salt, oleate, oxalate, pamoate (embonate), palmitate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide and valerate. Furthermore, where the compounds of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may
10 include alkali metal salts, e.g., sodium or potassium salts, alkaline earth metal salts, e.g., calcium or magnesium salts; and salts formed with suitable organic ligands, e.g., quaternary ammonium salts. All of the pharmacologically acceptable salts may be prepared by conventional means. (See Berge et al., J Pharm. Sci., 66(1), 1-19 (1977) for additional
15 examples of pharmaceutically acceptable salts.)

The compounds of the present invention can have chiral centers and occur as racemates, racemic mixtures, diastereomeric mixtures, and as individual diastereomers or enantiomers, with all isomeric forms included in the present invention. Therefore, where a compound is chiral, the separate
20 enantiomers or diastereomers, substantially free of the other, are included within the scope of the present invention; further included are all mixtures of the enantiomers or diastereomers. Also included within the scope of the invention are polymorphs, or hydrates or other modifiers of the compounds of invention.

25 The present invention includes within its scope prodrugs of the compounds of this invention. In general, such prodrugs will be functional derivatives of the compounds of this invention which are readily convertible *in vivo* into the required compound. For example, prodrugs of a carboxylic acid may include an ester, an amide, an ortho-ester, or heterocycles such
30 as tetrazole. Thus, in the methods of treatment of the present invention, the term "administering" shall encompass the treatment of the various conditions described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to the patient.
35 Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in "Design of Prodrugs," ed. H. Bundgaard, Elsevier, 1985, which is incorporated by reference herein in

TRANSGENIC SEEDS

5 its entirety. Metabolites of these compounds include active species produced upon introduction of compounds of this invention into the biological milieu.

For the selective inhibition or antagonism of $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ integrins, compounds of the present invention may be administered orally, 10 parenterally, or by inhalation spray, or topically in unit dosage formulations containing conventional pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes, for example, subcutaneous, intravenous, intramuscular, intrasternal, transmuscular infusion techniques or intraperitoneally.

15 The compounds of the present invention are administered by any suitable route in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended. Therapeutically effective doses of the compounds required to prevent or arrest the progress of or to treat the medical condition are readily ascertained by one of 20 ordinary skill in the art using preclinical and clinical approaches familiar to the medicinal arts.

Accordingly, the present invention provides a method of treating conditions mediated by selectively inhibiting or antagonizing the $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ cell surface receptor which method comprises administering a 25 therapeutically effective amount of a compound selected from the class of compounds depicted in the above formulas, wherein one or more compound is administered in association with one or more non-toxic, pharmaceutically acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as "carrier" materials) and if desired other 30 active ingredients. The present invention also provides a method for selective inhibition of the $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ cell surface receptors with a reduced $\alpha_v\beta_6$ inhibition. Another aspect of the invention provides a method for inhibiting bone resorption, treating osteoporosis, inhibiting humoral hypercalcemia of malignancy, treating Paget's disease, inhibiting tumor 35 metastasis, inhibiting neoplasia (solid tumor growth), inhibiting angiogenesis including tumor angiogenesis, treating retinopathy including macular degeneration and diabetic retinopathy, inhibiting arthritis, psoriasis

5 and periodontal disease, and inhibiting smooth muscle cell migration including restenosis.

In another embodiment, the present invention provides a method for selective antagonism of the $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ cell surface receptors over $\alpha_{IIb}\beta_3$, and in a further embodiment, also over the $\alpha_v\beta_6$ integrin receptor. Evidence of the toxicity of β_6 integrin antagonism indicates that it may be beneficial to spare antagonism of β_6 when designing $\alpha_v\beta_3$ antagonists, in addition to sparing $\alpha_{IIb}\beta_3$. Selectivity inhibition refers to a selectivity ratio of at least 10, more preferably 50, and even more preferably of at least 100. Selectivity ratio refers to the 10 selectivity of the IC_{50} of $\alpha_v\beta_6$ or $\alpha_{IIb}\beta_3$ over the selectivity of the IC_{50} of β_3 .

15 Based upon standard laboratory experimental techniques and procedures well known and appreciated by those skilled in the art, as well as comparisons with compounds of known usefulness, the compounds of Formula I or II can be used in the treatment of patients suffering from the 20 above pathological conditions. One skilled in the art will recognize that selection of the most appropriate compound of the invention is within the ability of one with ordinary skill in the art and will depend on a variety of factors including assessment of results obtained in standard assay and animal models.

25 Treatment of a patient afflicted with one of the pathological conditions comprises administering to such a patient an amount of compound of the Formula I which is therapeutically effective in controlling the condition or in prolonging the survivability of the patient beyond that expected in the absence of such treatment. As used herein, the term 30 "inhibition" of the condition refers to slowing, interrupting, arresting or stopping the condition and does not necessarily indicate a total elimination of the condition. It is believed that prolonging the survivability of a patient, beyond being a significant advantageous effect in and of itself, also indicates that the condition is beneficially controlled to some extent.

35 As stated previously, the compounds of the invention can be used in a variety of biological, prophylactic or therapeutic areas. It is contemplated

5 that these compounds are useful in prevention or treatment of any disease state or condition wherein the $\alpha_v \beta_3$ and/or $\alpha_v \beta_5$ integrin plays a role.

The dosage regimen for the compounds and/or compositions containing the compounds is based on a variety of factors, including the type, age, weight, sex and medical condition of the patient; the severity of
10 the condition; the route of administration; and the activity of the particular compound employed. Thus the dosage regimen may vary widely. Dosage levels of the order from about 0.01 mg to about 100 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions.

15 Oral dosages of the present invention, when used for the indicated effects, will range between about 0.01 mg per kg of body weight per day (mg/kg/day) to about 100 mg/kg/day, preferably 0.01 to 10 mg/kg/day, and most preferably 0.1 to 1.0 mg/kg/day. For oral administration, the compositions are preferably provided in the form of tablets containing 0.01,
20 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 200 and 500 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, preferably, from about 1mg to about 100 mg of active ingredient. Intravenous doses will
25 range from about 0.1 to about 10 mg/kg/minute during a constant rate infusion. Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds of the present invention can be administered in intranasal form via topical use of
30 suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration may be continuous rather than intermittent throughout the dosage regimen.

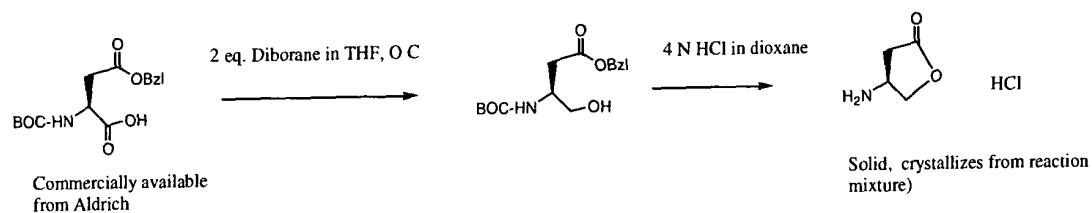
35 For administration to a mammal in need of such treatment, the compounds in a therapeutically effective amount are ordinarily combined with one or more adjuvants appropriate to the indicated route of

5 administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, gelatin, acacia, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or
10 encapsulated for convenient administration. Alternatively, the compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

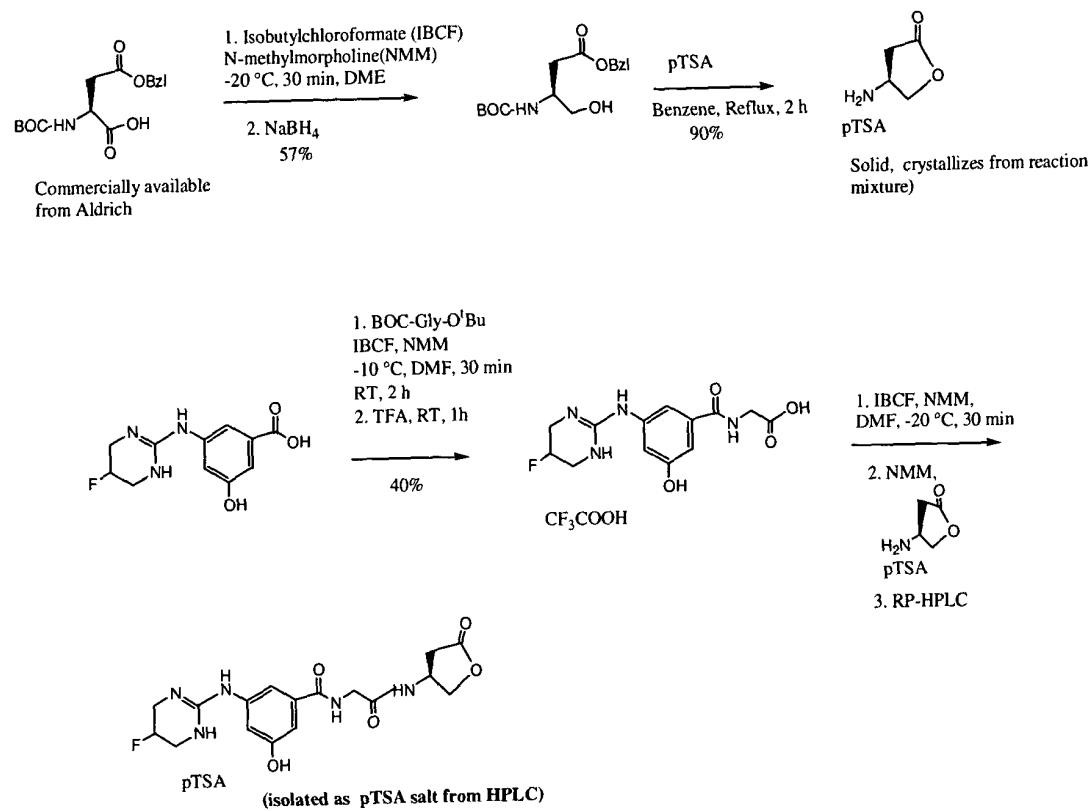
15 The pharmaceutical compositions useful in the present invention may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional pharmaceutical adjuvants such as preservatives, stabilizers, wetting agents, emulsifiers, buffers, etc.

20 The following Schemes are intended to be merely illustrative of the present invention, and not limiting thereof in either scope or spirit. Those with skill in the art will readily understand that known variations of the conditions and processes described in the Schemes can be used to make the embodiments of the invention.

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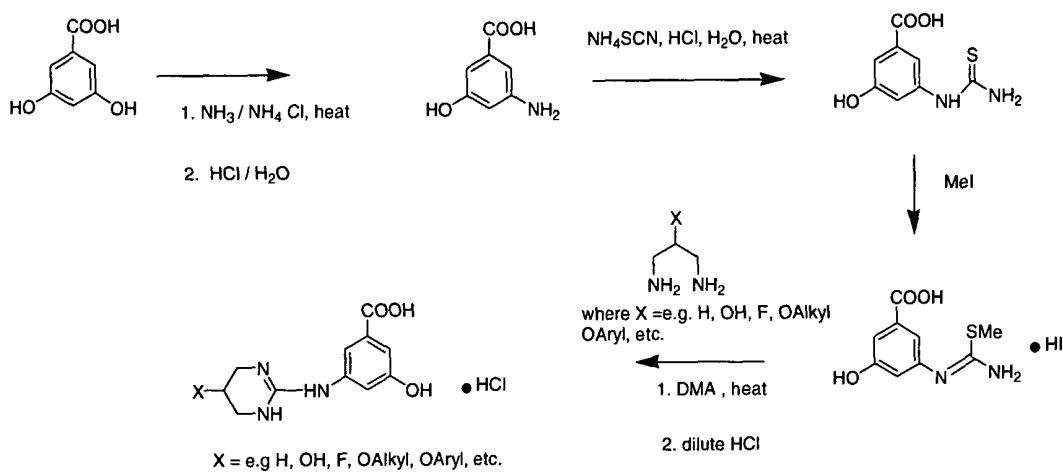
Scheme 1Preparation of S - 3 - aminobutyrolactone hydrochloride saltTOP SECRET//
REF ID: A6543950

Scheme 2



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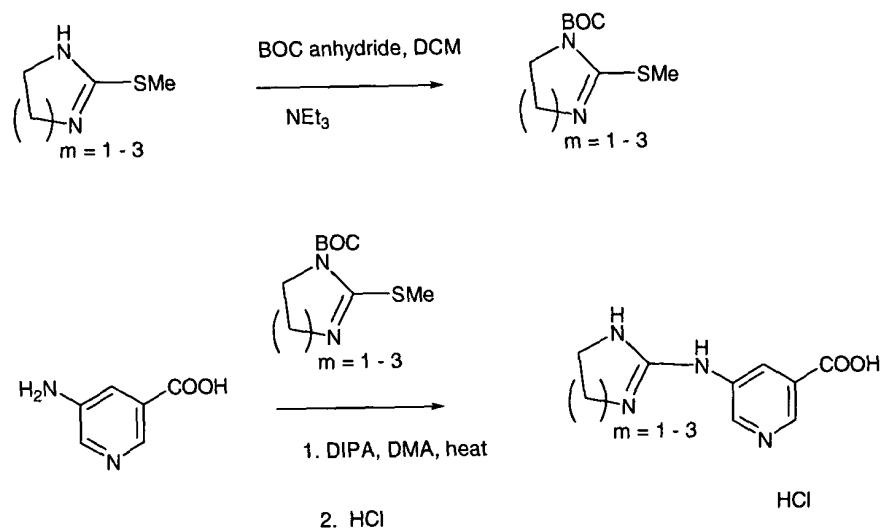
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SCHEME 3

05963627 092604

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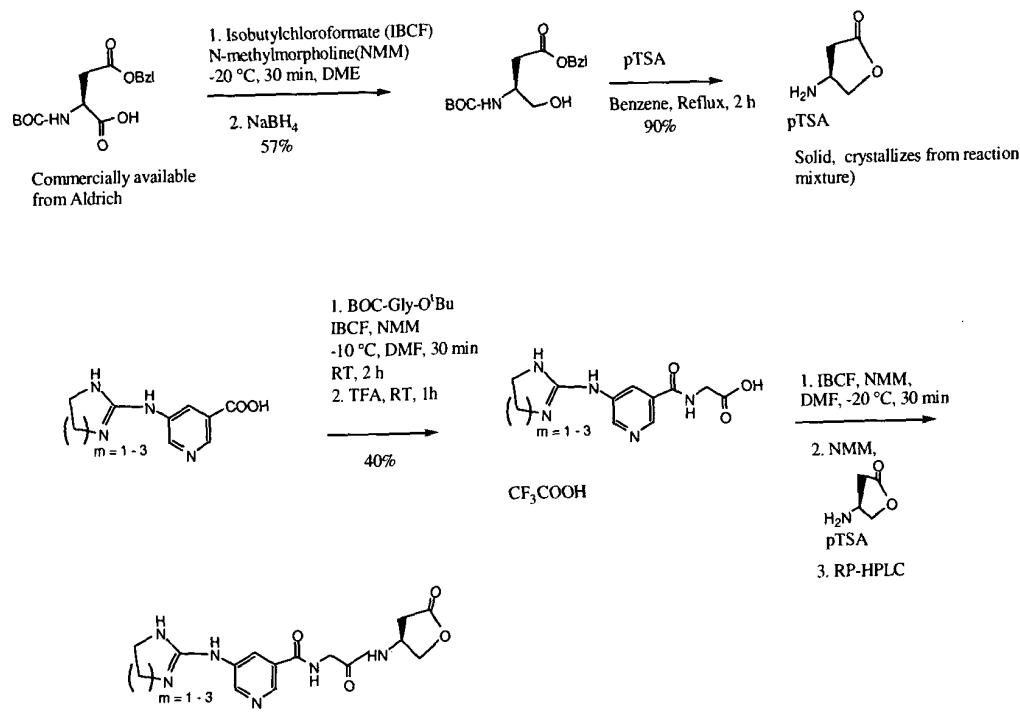
Scheme 4



4092610-AZ264265650

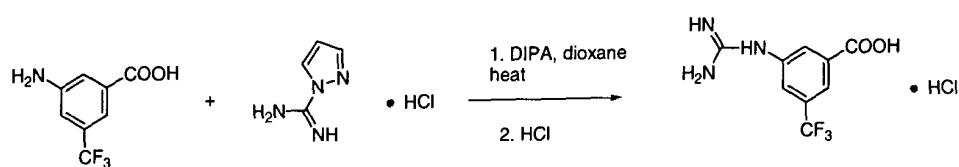
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Scheme 5



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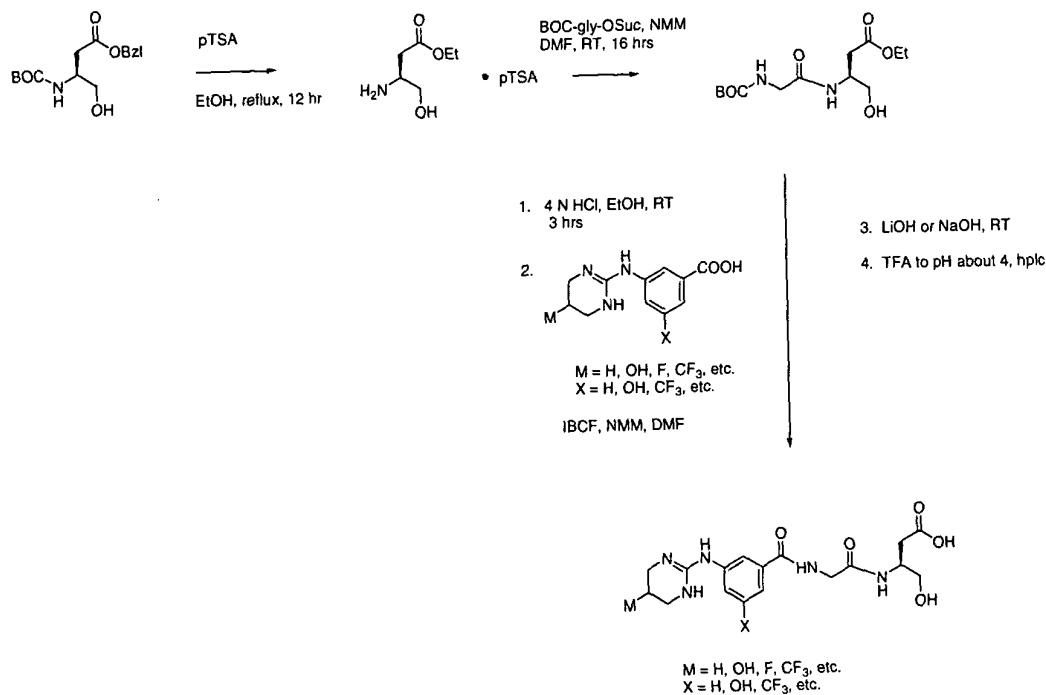
Scheme 6



452262 "09204

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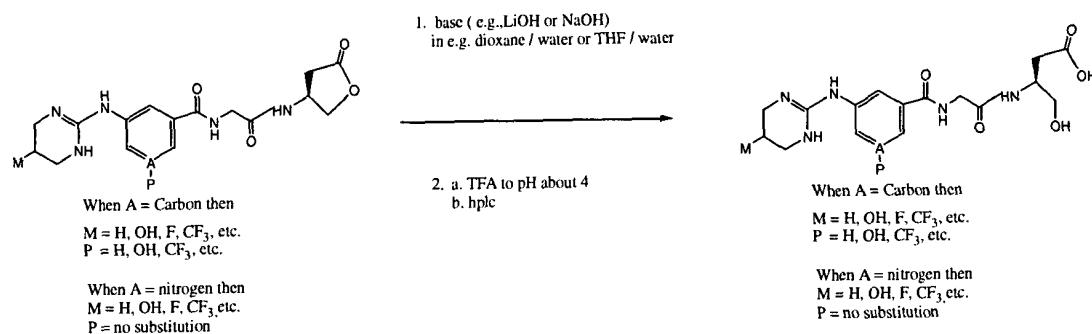
Scheme 7



T033360 - 200903080800

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Scheme 8



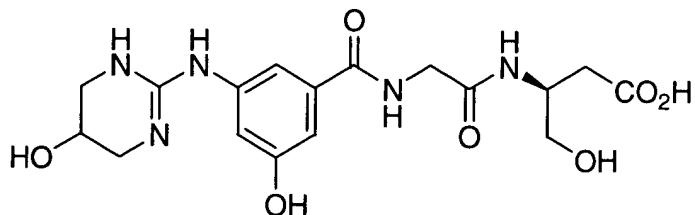
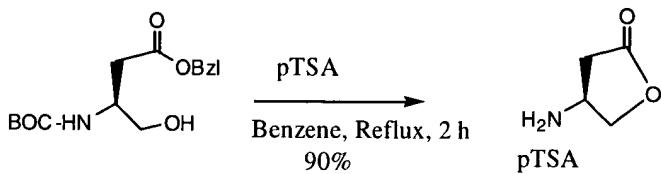
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The following Examples are intended to be illustrative and not intended to limit the scope of the invention.

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5

EXAMPLE 1Ex 1a

10

Solid, crystallizes from reaction mixture)

Synthesis of 3-amino-5-oxo-3S-furan hydrochloride

A solution of the Boc-aspartimol- γ -benzylester (0.5 g, Tetrahedron Lett .32, (7), 923, 1991) in dry benzene (10 .0 mL) containing p-toluenesulfonic acid (0.32 g) was heated to reflux for 1.5 h. under anhydrous conditions. The reaction mixture was cooled, diluted with ether and filtered the precipitate. It was washed with ether, and dried to give 0.38 g of the desired lactone as its p-toluenesufonate salt: $^1\text{H-NMR}$ (DMSO-d₆) δ 8.14 (br, 2H), 7.46 (d, 2H, J = 8.0 Hz), 7.09 (d, 2H, J = 8.0 Hz), 4.46 (m, 1H), 4.24 (m, 1H), 4.19(m, 1H), 2.96 (dd, 1H), 2.47 (dd, 2H), 2.2 (, 3H); MS: m/z 102 (MH⁺)

Ex 1bAlternate preparation of 3-amino-5-oxo-3S-furan hydrochloride.

N-tBoc-L-aspartic acid, β -benzyl ester (10.0 mmole) was dissolved in 10 mL of tetrahydrofuran (THF) and added drop-wise over a period of 30 min to a 0° C. solution of BH₃-THF (20 mL, 20.0 mmole) under argon. After the mixture was stirred for an additional 1-2 hr at 0° C, the reaction was quenched by drop-wise addition of 10% acetic acid in methanol and

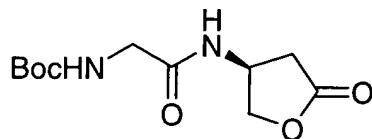
5 the solvent evaporated. The oil residue was dissolved in ethyl acetate and extracted with 1N HCl, water, and 1M NH₄HCO₃. The ethyl acetate layer was dried (Na₂SO₄) and volatiles evaporated to give an oil that could be crystallized from isopropanol/hexane (mp 56-57° C):¹H NMR (CDCl₃) δ 1.45 (s, 9H), 2.65 (d, 2H), 3.68 (d, 2H), 5.12 (s, 2H), 5.25 (m, 1H), 7.35 (m, 5H).

10 The resulting 3-N-tBoc-amino-4-hydroxy-butyric acid benzyl ester (20 g, 64 mmole) was stirred in 200 mL dichloromethane at room temperature for 16 hr in the presence of a catalytic amount of camphor sulfonic acid. Solvent was removed in vacuo and the crude product purified by flash chromatography (Merck 60 silica gel, ethyl acetate/hexane/1% 15 triethylamine). The N-tBoc-3-aminolactone was isolated as a white solid (5.4 g).

The 3-N-tBoc-aminolactone (5.0 g, 25 mmole) isolate was dissolved in 20 mL 4N HCl/dioxane. After 45 minutes at 25° C, 10 mL of 4N HCl/dioxane was added and after 1 hr the excess HCl was removed in 20 vacuo. The resulting solution deposited white crystals upon standing. These were filtered and dried to give 2.9 g of the desired product as the hydrochloride salt. ¹H NMR (d₆ DMSO) δ? 2.55 (dd, J₁=18.3 Hz, J₂=2.5 Hz), 3.0 (dd, 1H, J₁=8.5 Hz, J₂=18.3 Hz), 4.1 (m, 1H), 4.35 (dd, 1H, J₁=10.5 Hz, J₂=2.7 Hz), 4.5 (dd, 1H, J₁=10.5 Hz, J₂=6.5 Hz); MS (FAB) 102.1 (M +H).

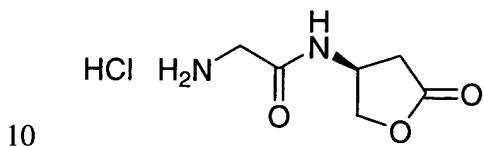
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Ex 1c



30 3-amino-5-oxo-3S-furan hydrochloride (2.9 g, 21 mmol) was dissolved in DMF (123 mL) and cooled to 0°C under a nitrogen atmosphere. N-Boc-Gly-OSu (5.4 g, 20 mmol) was added, followed by 4-methylmorpholine (2.3 mL, 21 mmol). After the solution was stirred 18 hours, it was diluted with brine and extracted twice with EtOAc. The combined organic layers were washed with H₂O, dried (Na₂SO₄), filtered, 35 and concentrated *in vacuo*. The residue was purified by chromatography

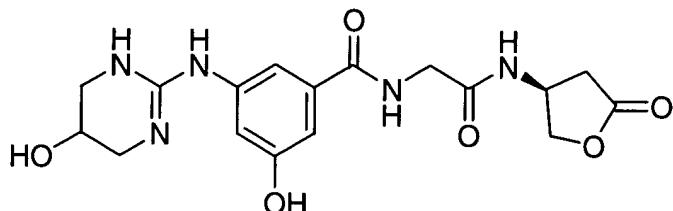
5 on silica gel (eluent: EtOAc) to give a colorless oil (4.7 g, 87%). ^1H NMR (CDCl_3) δ 1.46 (s, 9H), 2.51 (dd, 1H), 2.89 (dd, 1H), 3.82 (s, 2H), 4.25 (dd, 1H), 4.54 (dd, 1H), 4.72 (m, 1H).

Ex 1d

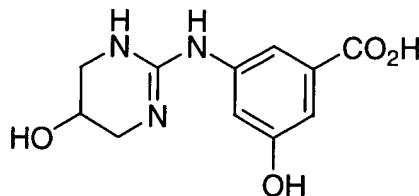
The colorless oil from Ex 1c (3.2 g, 12.4 mmol) was dissolved in 4N HCl dioxane (30 mL). After stirring 2.5 hours at ambient temperature, the excess HCl was removed *in vacuo*. The white solid material was filtered and dried (2.4 g, 98%). ^1H NMR (CD_3OD) δ 2.51 (dd, 1H), 2.92 (dd, 1H), 3.68 (s, 2H), 4.28 (dd, 1H), 4.57 (dd, 1H), 4.63 (m, 1H). EI-MS m/z 159 (MH^+).

15

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Ex 1e

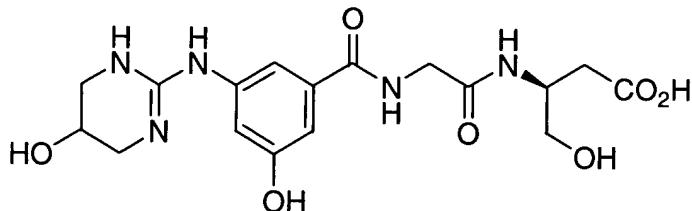
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**ACID A**

5 The amine hydrochloride from Ex 1d (950 mg, 4.9 mmol) and Acid A
 (1.4 g, 4.9 mmol; prepared according to US patent 6,013,651, Example H)
 were combined and slurried in DMF/CH₂Cl₂ (12 mL, 1:1) at ambient
 temperature under a nitrogen atmosphere. 1,3-Diisopropylcarbodiimide
 (0.9 mL, 5.9 mmol) was added, followed by 4-methylmorpholine (0.5 mL,
 10 4.9 mmol). After stirring 18 hours, the solution was filtered through a pad of
 Celite and the filtrate concentrated *in vacuo*. The resulting oil was purified
 by reverse-phase HPLC (H₂O/CH₃CN) to afford the title compound as a
 white solid (671 mg, 20%). ¹H NMR (DMSO-d₆) δ 2.37 (dd, 1H), 2.90 (dd,
 1H), 3.16 (dd, 2H), 3.35 (dd, 2H), 3.82 (d, 2H), 4.09 (m, 2H), 4.48 (m, 2H),
 15 6.75 (t, 1H) 7.11 (t, 1H), 7.14 (m, 1H), 8.18 (s, 2H), 8.54 (d, 1H), 8.63 (t,
 1H), 9.71 (s, 1H). EI-MS *m/z* 392 (MH⁺). Anal. Calcd for C₁₇H₂₁N₅O₆ +2.5
 TFA +0.5 H₂O: C, 38.55; H, 3.60. Found: C, 38.55; H, 3.87.

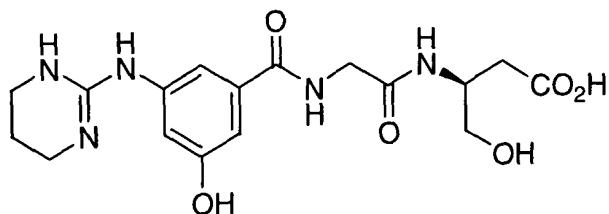
Ex 1f

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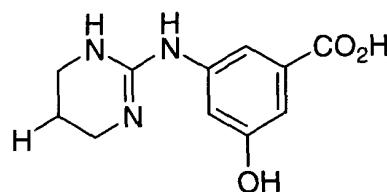
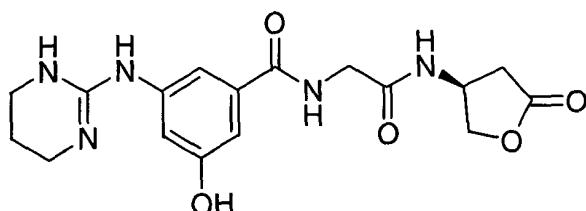


The desired hydroxy-acid was obtained by dissolving the product of
 Ex 1e (200 mg, 0.29 mmol) in water (7 mL). The pH of the solution was
 brought to about 11 by addition of dilute aqueous NaOH. Upon completion
 25 of the reaction, as determined by analytical reverse-phase HPLC, the
 solution was brought to a pH of about 8 by addition of TFA and
 concentrated *in vacuo*. Purification of the resulting residue by reverse-
 phase HPLC (H₂O/CH₃CN) afforded the title compound as a white solid (50
 mg, 17%). ¹H NMR (CD₃OD) δ 2.43 (dd, 1H), 2.55 (dd, 1H), 3.22 (dd, 2H),
 30 3.35 (dd, 2H), 3.49 (m, 2H), 3.92 (d, 2H), 4.12 (m, 1H), 4.21 (m, 1H), 6.74
 (t, 1H) 7.07 (t, 1H), 7.12 (m, 1H). EI-MS *m/z* 410 (MH⁺). Anal. Calcd for
 C₁₇H₂₃N₅O₇ +1.5 TFA +0.1 EtOAc: C, 41.58; H, 4.33; N, 11.89. Found: C,
 41.43; H, 4.21; N, 11.61.

5

EXAMPLE 2Ex 2a

10

**ACID B**

15 The amine hydrochloride from Ex 1d (950 mg, 4.9 mmol) and Acid B (1.3 g, 4.9 mmol; prepared using similar procedure according to US patent 6,013,651, Example H) were combined and slurried in DMF/CH₂Cl₂ (12 mL, 1:1) at ambient temperature under a nitrogen atmosphere. 1,3-Diisopropyl-carbodiimide (0.9 mL, 5.9 mmol) was added, followed by 4-methyl-morpholine (0.5 mL, 4.9 mmol). After stirring 18 hours, the solution was filtered through a pad of Celite and the filtrate concentrated *in vacuo*. The resulting oil was purified by reverse-phase HPLC (H₂O/CH₃CN) to afford the title compound as a light yellow solid (760 mg, 25%). ¹H NMR (DMSO-d₆) δ 1.89 (m, 2H), 2.37 (dd, 1H), 2.89 (dd, 1H), 3.16 (dd, 2H), 3.28 (m, 4H), 3.84 (d, 2H), 4.09 (m, 1H), 4.48 (m, 2H), 6.75 (t, 1H) 7.11 (t, 1H), 7.14 (m, 1H), 8.32 (s, 2H), 8.54 (d, 1H), 8.63 (t, 1H), 9.89 (s, 1H). EI-MS m/z 376

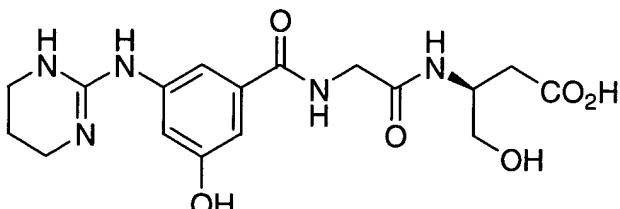
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25

5 (MH^+). Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{N}_5\text{O}_5 + 2 \text{TFA} + 0.3 \text{ H}_2\text{O}$: C, 41.43; H, 3.91; N, 11.50. Found: C, 41.21; H, 4.07; N, 11.74.

Ex 2b

10



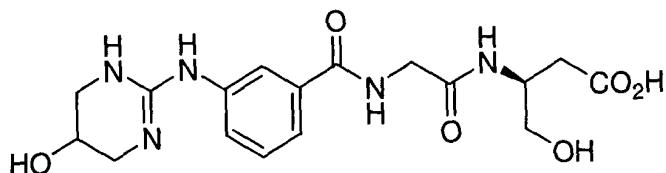
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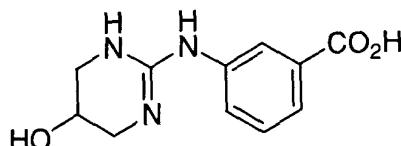
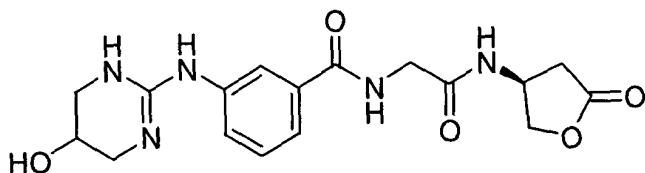
The desired hydroxy-acid was obtained by dissolving the lactone product of Ex 2a (200 mg, 0.29 mmol) in water (7 mL). The pH of the solution was brought to about 11 by addition of dilute aqueous NaOH. Upon completion of the reaction, as determined by analytical reverse-phase HPLC, the solution was brought to a pH of about 8 by addition of TFA and concentrated *in vacuo*. Purification of the resulting residue by reverse-phase HPLC ($\text{H}_2\text{O}/\text{CH}_3\text{CN}$) afforded the title compound as a white solid (50 mg, 17%). ^1H NMR (CD_3OD) δ 2.03 (m, 2H), 2.55 (dd, 1H), 2.67 (dd, 1H), 3.43 (m, 4H), 3.61 (m, 2H), 4.04 (d, 2H), 4.32 (m, 1H), 6.83 (t, 1H) 7.17 (t, 1H), 7.22 (m, 1H). EI-MS m/z 394 (MH^+). Anal. Calcd for $\text{C}_{17}\text{H}_{23}\text{N}_5\text{O}_6 + 3 \text{TFA}$: C, 37.56; H, 3.56; N, 9.52. Found: C, 36.37; H, 3.61; N, 9.80.

1000 900 800 700 600 500 400 300 200 100

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EXAMPLE 3Ex 3a

10

**15 ACID C**

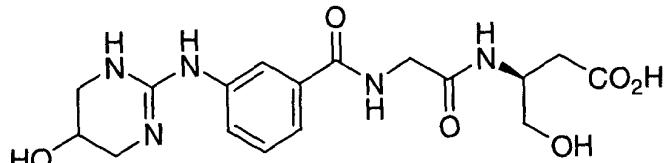
The amine hydrochloride from Ex 1d (950 mg, 4.9 mmol) and Acid C (1.0 g, 3.7 mmol; prepared using similar procedure according to US patent 6,013,651, Example H) were combined and slurried in DMF/CH₂Cl₂ (12 mL, 1:1) at ambient temperature under a nitrogen atmosphere. 1,3-Diisopropylcarbodiimide (0.9 mL, 5.9 mmol) was added, followed by 4-methylmorpholine (0.5 mL, 4.9 mmol). After stirring 18 hours, the solution was filtered through a pad of Celite and the filtrate concentrated *in vacuo*. The resulting oil was purified by reverse-phase HPLC (H₂O/CH₃CN) to afford the title compound as a light yellow solid (150 mg, 7%). ¹H NMR (DMSO-d₆) δ 2.39 (dd, 1H), 2.90 (dd, 1H), 3.17 (dd, 2H), 3.38 (dd, 2H), 3.87 (d, 2H), 4.10 (m, 2H), 4.48 (m, 2H), 7.36 (m, 1H), 7.52 (t, 1H), 7.70 (m, 1H), 7.75 (m, 1H), 8.38 (s, 2H), 8.62 (d, 1H), 8.83 (t, 1H), 10.08 (s, 1H). EI-MS

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5 m/z 376 (MH^+). Anal. Calcd for $C_{17}H_{21}N_5O_5 + 2$ TFA + 0.5 H_2O : C, 41.18; H, 3.95; N, 11.44. Found: C, 40.86; H, 3.90; N, 11.83.

Ex 3b

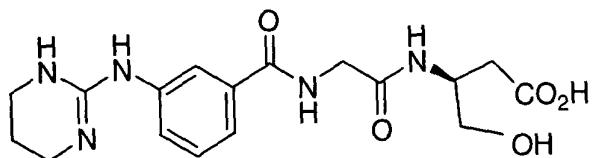
10



The desired hydroxy-acid was obtained by dissolving the lactone product of Ex 3a (200 mg, 0.29 mmol) in water (7 mL). The pH of the solution was brought to about 11 by addition of dilute aqueous NaOH.
15 Upon completion of the reaction, as determined by analytical reverse-phase HPLC, the solution was brought to a pH of about 8 by addition of TFA and concentrated *in vacuo*. Purification of the resulting residue by reverse-phase HPLC (H_2O/CH_3CN) afforded the title compound as a white solid (50 mg, 17%). 1H NMR ($DMSO-d_6$) δ 2.31 (dd, 1H), 2.52 (dd, 1H), 3.16 (m, 2H), 3.34 (m, 2H), 3.36 (m, 2H), 3.86 (d, 2H), 4.08 (m, 2H), 7.36 (m, 1H)
20 7.52 (t, 1H), 7.68 (m, 1H), 7.74 (m, 1H), 7.83 (d, 1H), 8.38 (s, 2H), 8.72 (t, 1H), 10.08 (s, 1H). EI-MS m/z 394 (MH^+). Anal. Calcd for $C_{17}H_{23}N_5O_6 + 7$ TFA + 1 $CH_3CN + 4 H_2O$: C, 30.38; H, 3.17; N, 6.44. Found: C, 30.19; H, 2.90; N, 6.69.

25

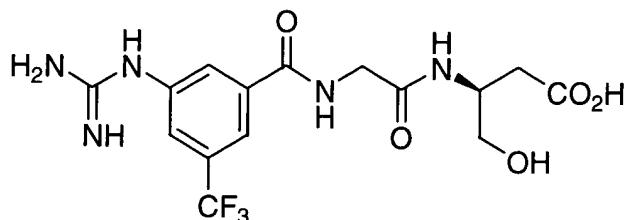
5

EXAMPLE 4

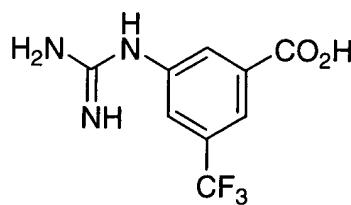
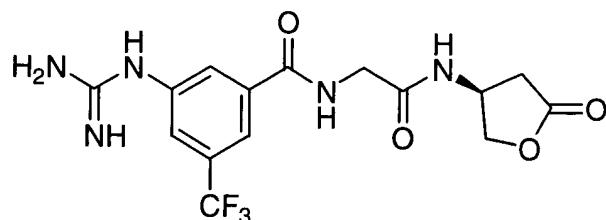
The title compound was prepared from the corresponding lactone using substantially the procedure described for **EXAMPLE 1** (30% yield). ¹H NMR (DMSO-d₆) δ 1.89 (m, 2H), 2.33 (dd, 1H), 2.43 (dd, 1H), 3.28 (m, 4H), 3.55 (m, 2H), 3.87 (d, 2H), 4.05 (m, 1H), 7.33 (dd, 1H), 7.51 (t, 1H), 7.69 (m, 1H), 7.72 (m, 1H), 7.89 (d, 1H), 8.48 (br s, 2H), 8.72 (t, 1H). EI-MS m/z (m, 1H), 7.72 (m, 1H), 7.89 (d, 1H), 8.48 (br s, 2H), 8.72 (t, 1H). Anal. Calcd for C₁₇H₂₃N₅O₅ +1TFA +1 DMF +2 H₂O: C, 44.00; 378 (MH⁺). Found: C, 44.13; H, 5.91; N, 13.10.

20

5

EXAMPLE 5Ex 5a

10

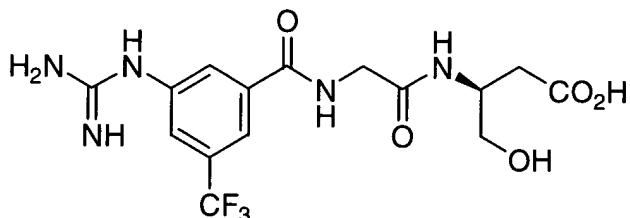
**ACID E**

The amine hydrochloride from Ex 1c (950 mg, 4.9 mmol) and Acid B (1.0 g, 3.5 mmol; prepared using similar procedure according to US patent 6,028,223, EXAMPLE 38) were combined and slurried in DMF/CH₂Cl₂ (12 mL, 1:1) at ambient temperature under a nitrogen atmosphere. 1,3-Diisopropylcarbodiimide (0.9 mL, 5.9 mmol) was added, followed by 4-methylmorpholine (0.5 mL, 4.9 mmol). After stirring 18 hours, the solution was filtered through a pad of Celite and the filtrate concentrated *in vacuo*. The resulting oil was purified by reverse-phase HPLC (H₂O/CH₃CN) to afford the title compound as a light yellow solid (600 mg, 30%). ¹H NMR (DMSO-d₆) δ 1.89 (m, 2H), 2.39 (dd, 1H), 2.89 (dd, 1H), 3.28 (m, 4H), 3.84 (d, 2H), 4.09 (m, 1H), 4.48 (m, 2H), 7.37 (m, 1H), 7.53 (t, 1H), 7.70 (m, 1H), 7.74 (m, 1H), 8.29 (s, 2H), 8.61 (d, 1H), 8.83 (t, 1H), 9.93 (s, 1H). EI-MS

1000 900 800 700 600 500 400 300 200 100

5 m/z 360 (MH^+). Anal. Calcd for $\text{C}_{17}\text{H}_{21}\text{N}_5\text{O}_4 + 1 \text{ TFA} + 1 \text{ H}_2\text{O} + 1 \text{ DMF}$: C, 46.81; H, 5.54; N, 14.89. Found: C, 46.69; H, 5.21; N, 14.69.

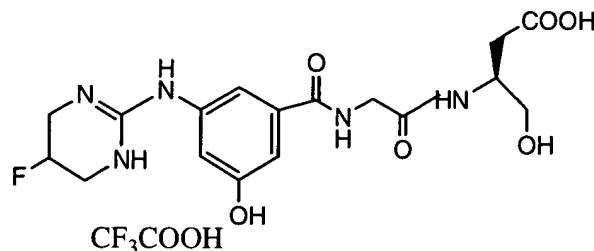
Ex 5b



10

The desired hydroxy-acid was obtained by dissolving the lactone product of Ex 5a (200 mg, 0.29 mmol) in water (7 mL). The pH of the solution was brought to about 11 by addition of dilute aqueous NaOH. Upon completion of the reaction, as determined by analytical reverse-phase HPLC, the solution was brought to a pH of about 8 by addition of TFA and concentrated *in vacuo*. Purification of the resulting residue by reverse-phase HPLC ($\text{H}_2\text{O}/\text{CH}_3\text{CN}$) afforded the title compound as a white solid (50 mg, 17%). ^1H NMR (CD_3OD) δ 2.55 (dd, 1H), 2.68 (dd, 1H), 3.62 (m, 2H), 4.08 (d, 2H), 4.33 (m, 1H), 7.78 (s, 1H), 8.06 (s, 1H), 8.18 (s, 1H). EI-MS *m/z* 406 (MH^+). Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{N}_5\text{O}_5\text{F}_3 + 1.5 \text{ TFA}$: C, 37.51; H, 3.41; N, 12.15. Found: C, 37.35; H, 3.24; N, 12.01.

5

EXAMPLE 6**Ex 6a**

Preparation of BOC-NH-CH(CH₂COOBzL)-CH₂-OH: This
10 compound was prepared according to the literature *Tet Letts.* 32, (7)923,
1991.

Ex 6b

A solution of Boc-aspartimol- γ -benzylester (1.0 g, 0.0032 mol) in
15 EtOH (10.0 mL) containing p-toluenesulfonic acid (0.65 g) was heated to
reflux for 12 h under anhydrous conditions. After removal of the solvent
under reduced pressure, the residue was dissolved in DMF (10.0 mL),
cooled in an ice bath, added N-methylmorpholine (0.6 mL), and BOC-Gly-
ONSu (0.9 g). The resulting mixture was stirred at room temperature for 16
20 h. The reaction mixture was then poured into cold water (25 mL) and
extracted with EtOAc (3 x 25 mL). The combined organic extracts were
washed with 5% citric acid (2 x 25 mL), water, (2 X 25 mL), dried (Na₂SO₄)
and concentrated to dryness. The resulting intermediate (0.75 g) was
dissolved in EtOH (5.0 mL), added 4N HCl/dioxane (5.0 mL), and stirred at
25 room temperature for 3 h. After removal of the solvents, the residue was
dried in a desiccator over NaOH pellets for 4 h, and used in the following
step without purification.

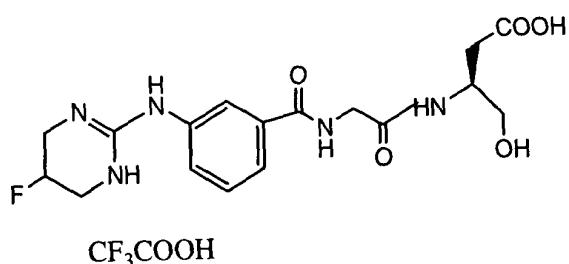
To a solution of (5-fluoro-tetrahydropyrimidine)-3-amino-5-
hydroxybenzoic acid (1.0 g, 0.00345 mol) in DMF (5.0 mL) at - 20 °C, was
30 added isobutylchloroformate (0.45 mL), followed by the addition of N-
methylmorpholine (0.4 mL). After stirring for 20 min, a solution of the free
amine generated by the addition of N-methylmorpholine (0.42 mL) to a
solution of the product from step B in DMF (5.0 mL) was added. The

5 resulting mixture was stirred at room temperature for 16 h. The solvents
were distilled in vacuo, and the product was purified by reverse-phase
HPLC using 10-90% acetonitrile/water at a flow rate of 70 mL/min. The
appropriate fractions (MH^+ m/z = 440) were combined and freeze dried to
obtain 0.22 g of the desired ester This material was stirred with 1N LiOH
10 (1.0 mL) for 1 h, diluted with water (5 .0 mL), cooled, and acidified with
trifluoroacetic acid. The resulting mixture was purified by reverse-phase
HPLC using 10-90% acetonitrile/water gradient (30 min) at flow rate of 70
mL/min. The appropriate fractions as revealed by mass spectrum of the
fractions (MH^+ m/z = 412) were combined and freeze dried to obtain the
desired acid (0.14 g) as its trifluoroacetate salt: $^1\text{H-NMR}$ (CD_3OD) δ ? 7.21
(m, 1H), 7.16 (t, 1H, J = 1.6 Hz), 6.82 (t, 1H, J = 1.6 Hz), 5.20 (d, 1H, $J_{\text{H},\text{F}}$ =
44 Hz, J = 2.4Hz), 4.26 (m, 1H), 3.99 (d, 2H, J = 1.2 Hz), 3.70- 3.45 (m,
6H), and 2.64 - 2.48 (2dd, 2H, J_1 = 6.4 Hz, J_2 = 16 Hz); HR-MS: m/z calcd
for $\text{C}_{17}\text{H}_{23}\text{N}_5\text{O}_6\text{F}$ (MH^+) 412.1632, found 412.1630.

20

1234567890

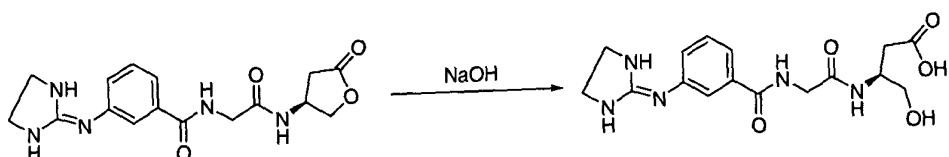
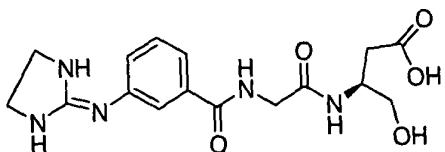
5

EXAMPLE 7

The title compound was prepared as described according to the preparation of EXAMPLE 6, substituting (5-fluoro-tetrahydropyrimidine)-3-amino benzoic acid for (5-fluoro-tetrahydropyrimidine)-3-amino-5-hydroxybenzoic acid. ¹H-NMR (CD₃OD) δ 7.82 (m, 1H), 7.74 (m, 1H), 7.58 (m, 1H), 7.42 (m, 1H), 5.21 (d, 1H, J_{H,F} = 46.4 Hz, J = 2.0Hz), 4.27 (m, 1H), 4.03 (q, 2H), 3.65 - 3.49 (m, 6H), 2.62 (dd, 1H, J = 6.2 Hz), 2.51 (dd, 1H, J = 6.2 Hz); HR-MS: m/z calcd for C₁₇H₂₃N₅O₅F (MH⁺) 396.1683, found 15 396.1710.

X092650 "262660

5

EXAMPLE 8

10

To the mixture of lactone (300 mg) in methanol (3.0 ml) and THF (3.0 ml) was added 1N NaOH solution (3.0 ml). After it was stirred for 2 hours at room temperature, the mixture was neutralized with 1N HCl solution (3.0 ml) and the solvents were removed under reduced pressure.

15

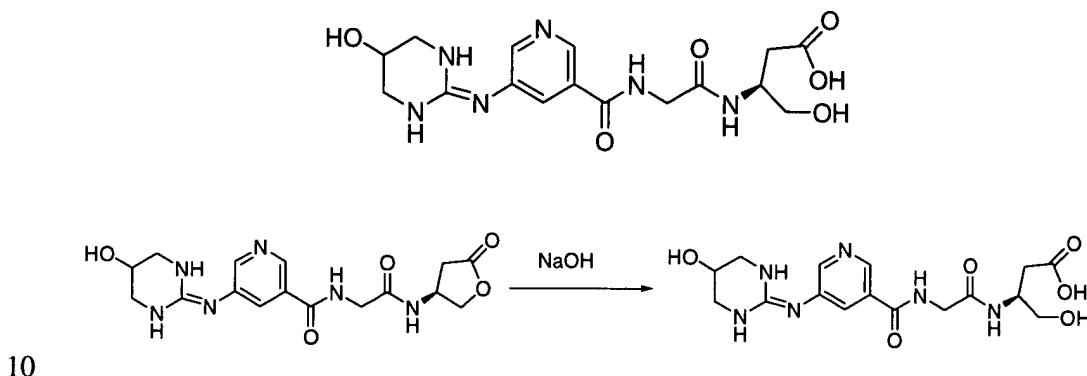
The residue was purified through preparative HPLC (C-18 column, eluted with water/acetonitrile) to yield 100 mg of the title compound as a white amorphous solid. ^1H NMR(400 MHz, DMSO-d₆, vs TMS) δ : 2.53(1H, dd, J = 7.21, 7.18 Hz), 2.66(1H, dd, J = 6.10, 6.22 Hz), 3.61(2H, m), 3.82(4H, s), 4.03(2H, d, J = 4.2 Hz), 4.32(1H, m), 7.47(1H, d, J = 6.96 Hz), 7.59(1H, t, J = 7.82 Hz), 7.79(1H, d, J = 1.75 Hz), 7.83(1H, d, J = 8.82 Hz) ppm. Anal.

20

Calcd for C₁₆H₂₁N₅O₅·0.5 H₂O·1.2TFA, C, 43.40; H, 4.59; N, 13.75. Found: C, 43.10; H, 4.43; N, 13.75.

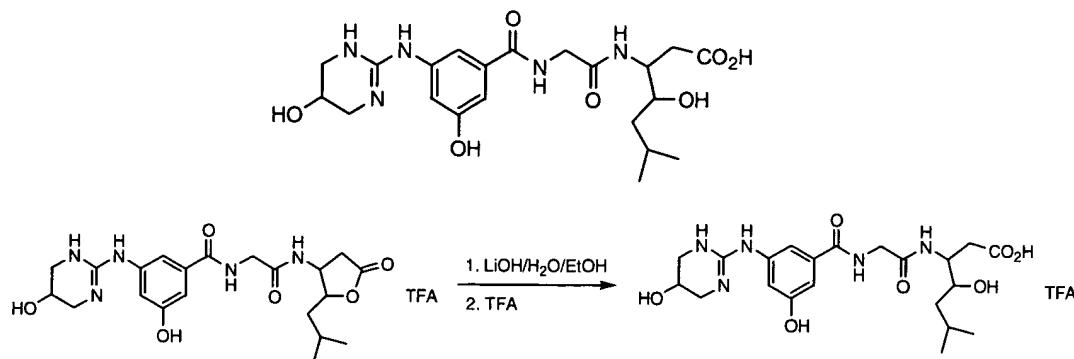
409260 "✓26E2610

5

EXAMPLE 9

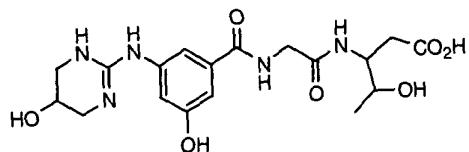
To the mixture of the lactone (250 mg) in methanol (2.0 ml) and THF (2.0 ml) was added 1N NaOH solution (2.0 ml). After it was stirred for 2 hours at room temperature, the mixture was neutralized with 1N HCl solution (3.0 ml) and the solvents were removed under reduced pressure. The residue was purified through preparative HPLC (C-18 column, eluted with water/acetonitrile) to yield 150 mg of the title compound as a white amorphous solid. ^1H NMR(400 MHz, DMSO-d₆, vs TMS) δ : 2.54(1H, dd, J = 7.27, 7.28 Hz), 2.64(1H, dd, J = 6.16, 6.17 Hz), 3.36(2H, dd, J = 3.20, 3.33 Hz), 3.52(2H, dd, J = 2.88, 2.88 Hz), 3.62(2H, t, J = 9.40 Hz), 4.09(2H, d, J = 4.27), 4.30(2H, m), 8.22(1H, m), 8.66(1H, d, J = 2.38 Hz), 8.95(1H, d, J = 1.70 Hz)ppm. HRMS(FAB): Exact Mass calcd for (C₁₆H₂₂N₆O₆ + H) = 395.1678. Found: (M+H) = 395.1688.

1000 2000 3000 4000 5000 6000 7000 8000 9000

EXAMPLE 10

10 To a stirring solution of the lactone (160 mg, 0.28 mmol) in a mixture of water and ethanol was added LiOH·H₂O (100 mg) at room temperature. After about 30 minutes, reaction went to completion. The basic solution was acidified to pH=1.0 then purified by reverse phase preparative HPLC right after acidification. Fractions were combined and frozen dried
 15 immediately after chromatography. ¹H NMR (400 MHZ, CD₃OD): δ (ppm) 0.88-0.92 (m, 6H), 1.18-1.25 (m, 1H), 1.32-1.39 (m, 1H), 1.72-1.79 (m, 1H), 2.49 (dd, *J*=7.2, 16.0 Hz, 1H), 2.66 (dd, *J*=6.8, 16.0 Hz, 1H), 3.30 (dd, *J*=3.6, 12.4 Hz, 2H), 3.44 (dd, *J*=2.8, 12.4 Hz, 2H), 3.76-3.79 (m, 1H), 3.99 (d, *J*=16.4 Hz, 1H), 4.04 (d, *J*=16.4 Hz, 1H), 4.21-4.29 (m, 2H), 6.82 (t, *J*=2.0 Hz, 1H), 7.17 (t, *J*=2.0 Hz, 1H), 7.19 (t, *J*=2.0 Hz, 1H); Theoretical
 20 MS (M+H): 466.2302; Found: 466.2312.

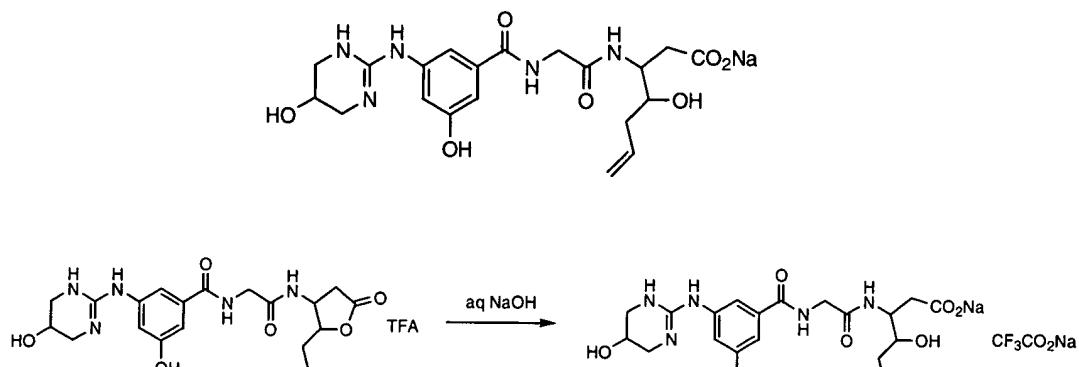
TRIGENIC PHARMA INC.
 1000 CHAMBERS ST.
 SUITE 1000
 HOUSTON, TX 77002
 U.S.A.

EXAMPLE 11

10 The title compound was prepared from the corresponding lactone according to the procedure described for the preparation of **EXAMPLE 10**.
15 ^1H NMR (400 MHZ, D_2O): δ (ppm) 1.00 (d, $J=6.4$ Hz, 3H), 2.42 (dd, $J=9.6$, 16.0 Hz, 1H), 2.54 (dd, $J=5.2$, 16.0 Hz, 1H), 3.22 (dd, $J=3.2$, 13.2 Hz, 2H), 3.35 (dd, $J=2.0$, 13.2Hz, 2H), 3.76-3.79 (m, 1H), 3.65 (d, $J=16.8$ Hz, 1H), 3.97 (d, $J=16.8$ Hz, 1H), 4.08-4.11 (m, 1H), 4.22-4.24 (m, 1H), 6.82 (t, $J=2.0$ Hz, 1H), 7.09 (brs, 2H); Theoretical MS ($\text{M}+\text{H}$): 424.1832; Found: 424.1823.

1234567890-2345678901234567890

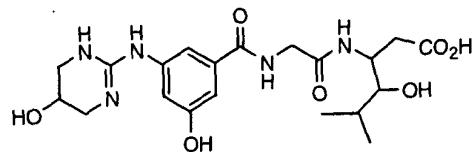
5

EXAMPLE 12

The lactone (51.4 mg; 0.094 mmol) was treated with 0.3 M aqueous sodium hydroxide (0.629 mL, 0.188 mmol) at room temperature. The progress of reaction was monitored by mass spectrometry. The mixture was frozen dried when reaction completed to give a light yellow solid.

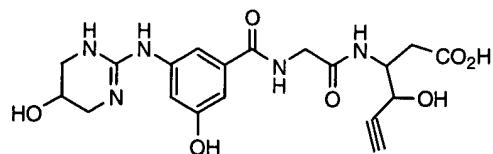
¹H NMR (400 MHZ, D₂O): δ (ppm) 1.98-2.07 (m, 1H), 2.14-2.23 (m, 1H), 2.20 (dd, *J*=9.2, 14.8 Hz, 1H), 2.40 (dd, *J*=4.8, 14.8 Hz, 1H), 3.22 (dd, *J*=3.2, 12.8 Hz, 2H), 3.35 (dd, *J*=2.8, 12.8 Hz, 2H), 3.57-3.62 (m, 1H), 3.89 (d, *J*=16.8 Hz, 1H), 3.94 (d, *J*=16.8 Hz, 1H), 4.04-4.09 (m, 1H), 4.21-4.23 (m, 1H), 4.95-5.03 (m, 2H), 5.67-5.78 (m, 1H), 6.56 (t, *J*=2.0 Hz, 1H), 6.77 (t, *J*=2.0 Hz, 1H), 6.84 (t, *J*=2.0 Hz, 1H); Theoretical MS (M+H): 450.1989; Found: 450.1987.

5

EXAMPLE 13

10 The title compound was prepared from the corresponding lactone according to the procedure described for the preparation of **EXAMPLE 10**.
15 ^1H NMR (400 MHZ, CD₃OD): δ (ppm) 0.91 (d, $J=6.4$ Hz, 3H), 0.96 (d, $J=6.4$ Hz, 3H), 1.59-1.65 (m, 1H), 2.51 (dd, $J=2.4, 16.0$ Hz, 1H), 2.64 (dd, $J=7.2, 16.0$ Hz, 1H), 3.21 (dd, $J=1.6, 8.4$ Hz, 1H), 3.31 (d, $J=3.6, 12.0$ Hz, 2H), 3.44 (dd, $J=2.4, 12.0$ Hz, 2H), 4.00 (t, $J=6.8$ Hz, 2H), 4.21-4.23 (m, 1H), 4.46-4.50 (m, 1H), 6.82 (t, $J=2.0$ Hz, 1H), 7.17 (t, $J=2.0$ Hz, 1H), 7.19 (t, $J=2.0$ Hz, 1H); Theoretical MS (M+H): 452.2150; Found: 452.2145.

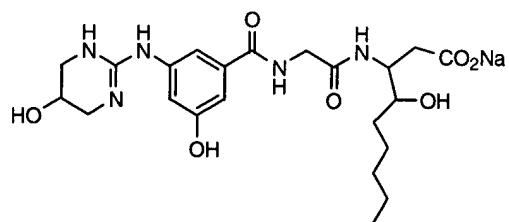
FOREGO X25E950

EXAMPLE 14

The title compound was prepared from the corresponding lactone
10 according to the procedure described for the preparation of **EXAMPLE 10**.
 ^1H NMR (300 MHZ, CD₃OD): δ (ppm) 2.62 (dd, $J=8.7, 16.2$ Hz, 1H), 2.84 (dd, $J=4.8, 16.2$ Hz, 1H), 2.88 (s, 1H), 3.34 (dd, $J=3.6, 12.3$ Hz, 2H), 3.49 (dd, $J=2.7, 12.3$ Hz, 2H), 4.09 (t, $J=3.6$ Hz, 2H), 4.25-4.27 (m, 1H), 4.39-4.48 (m, 1H), 4.53-4.55 (m, 1H), 6.86 (t, $J=2.1$ Hz, 1H), 7.20 (t, $J=2.1$ Hz, 1H), 7.23 (t, $J=2.1$ Hz, 1H); Theoretical MS (M+H): 434.1676; Found: 15 434.1673.

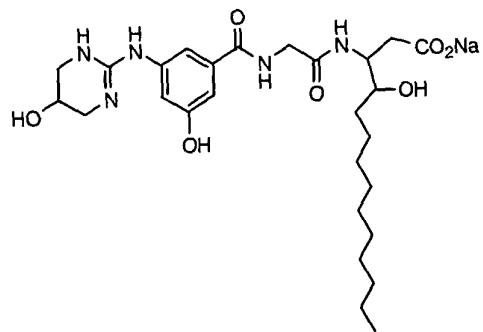
T0033614 20200629 0650

EXAMPLE 15



The title compound was prepared from the corresponding lactone according to the procedure described for the preparation of **EXAMPLE 10**.
¹H NMR (300 MHZ, CD₃OD): δ (ppm) 0.92 (t, *J*=6.6 Hz, 3H), 1.33-1.47 (m, 8H), 2.53 (dd, *J*=7.2, 15.9 Hz, 1H), 2.69 (dd, *J*=6.9, 15.9 Hz, 1H), 3.34 (dd, *J*=3.3, 12.3 Hz, 2H), 3.48 (dd, *J*=2.7, 12.3 Hz, 2H), 3.65-3.72 (m, 1H), 4.02 (d, *J*=16.5 Hz, 1H), 4.08 (d, *J*=16.5 Hz, 1H), 4.25-4.29 (m, 1H), 4.29-4.39 (m, 1H) 6.86 (t, *J*=2.1 Hz, 1H), 7.22 (t, *J*=2.1 Hz, 1H), 7.24 (t, *J*=2.1 Hz, 1H); Theoretical MS (M+H): 480.2458; Found: 480.2453.

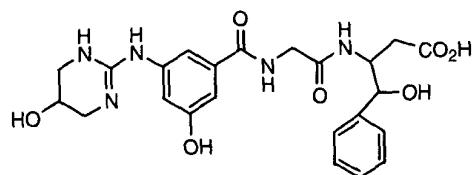
5

EXAMPLE 16

The title compound was prepared from the corresponding lactone
10 according to the procedure described for the preparation of **EXAMPLE 10**.
¹H NMR (400 MHZ, CD₃OD): δ (ppm) 0.85-0.91 (m, 3H), 1.20-1.48 (m,
18H), 2.49 (dd, *J*=7.2, 15.6 Hz, 1H), 2.64 (dd, *J*=6.4, 15.6 Hz, 1H), 3.31
(dd, *J*=3.2, 12.4 Hz, 2H), 3.44 (dd, *J*=2.4, 12.4 Hz, 2H), 3.62-3.68 (m, 1H),
15 4.00 (d, *J*=12.8 Hz, 1H), 4.03 (d, *J*=12.8 Hz, 1H), 4.21-4.23 (m, 1H), 4.25-
4.32 (m, 1H), 6.82 (t, *J*=2.0 Hz, 1H), 7.17 (t, *J*=2.1 Hz, 1H), 7.19 (t, *J*=2.1
Hz, 1H); Theoretical MS (M+H): 550.3241; Found: 550.3233.

100-000-20000000

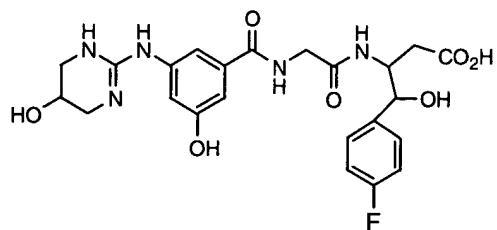
5

EXAMPLE 17

The title compound was prepared from the corresponding lactone
10 according to the procedure described for the preparation of **EXAMPLE 10**.
¹H NMR (400 MHZ, CD₃OD): δ (ppm) 2.47 (dd, *J*=4.4, 16.4 Hz, 1H), 2.55
(dd, *J*=8.8, 16.4 Hz, 1H), 3.31 (dd, *J*=3.6, 12.8 Hz, 2H), 3.45 (dd, *J*=2.8,
12.8 Hz, 2H), 3.92 (d, *J*=16.8 Hz, 1H), 3.97 (d, *J*=16.8 Hz, 1H), 4.21-4.23
(m, 1H), 4.42-4.47 (m, 1H), 6.82 (t, *J*=2.4 Hz, 1H), 7.16 (t, *J*=2.4 Hz, 1H),
15 7.18 (t, *J*=2.4 Hz, 1H), 7.18-7.40 (m, 5H); Theoretical MS (M+H): 486.1989;
Found: 486.1983.

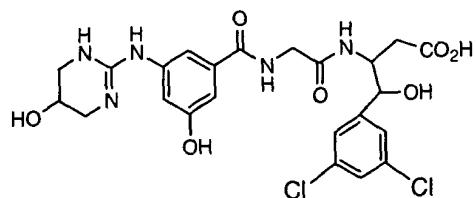
SEARCHED = 20060822 00:00:00
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EXAMPLE 18



The title compound was prepared from the corresponding lactone according to the procedure described for the preparation of **EXAMPLE 10**.
 10 ^1H NMR (400 MHZ, CD₃OD): δ (ppm) 2.38 (dd, $J=7.6, 16.0$ Hz, 1H), 2.72 (dd, $J=6.0, 16.0$ Hz, 1H), 3.32 (dd, $J=3.6, 12.4$ Hz, 2H), 3.44 (dd, $J=2.4, 12.4$ Hz, 2H), 3.93 (t, $J=4.4$ Hz, 2H), 4.21-4.23 (m, 1H), 4.43-4.50 (m, 1H), 6.82 (t, $J=2.0$ Hz, 1H), 6.98-7.03 (m, 2H), 7.15 (t, $J=2.0$ Hz, 1H), 7.18 (t, 15 $J=2.0$ Hz, 1H), 7.36-7.39 (m, 2H); Theoretical MS (M+H): 504.1895; Found: 504.1893.

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EXAMPLE 19

The title compound was prepared from the corresponding lactone according to the procedure described for the preparation of **EXAMPLE 10**.

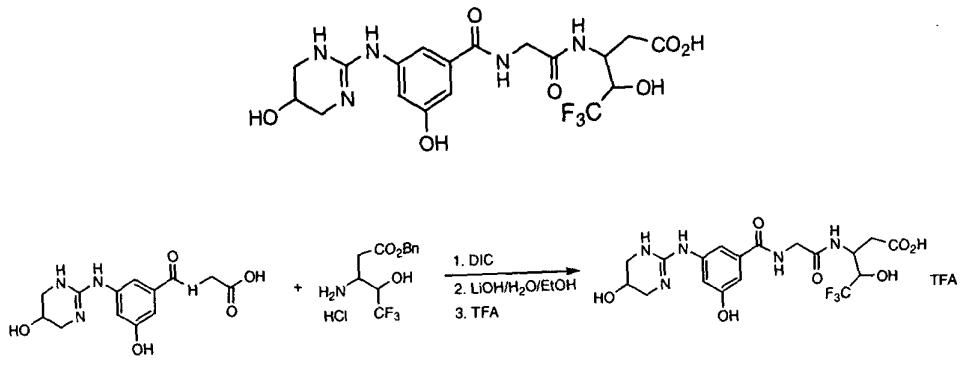
Diastereomer **a**): ^1H NMR (400 MHZ, CD₃OD): δ (ppm) 2.44 (dd, $J=6.8, 16.0$ Hz, 1H), 2.77 (dd, $J=6.8, 16.0$ Hz, 1H), 3.30 (dd, $J=3.6, 12.4$ Hz, 2H), 3.44 (dd, $J=2.8, 12.4$ Hz, 2H), 3.87 (d, $J=16.4$ Hz, 1H), 3.97 (d, $J=16.4$ Hz, 1H), 4.21-4.23 (m, 1H), 4.47-4.51 (m, 1H), 6.81 (t, $J=2.0$ Hz, 1H), 7.15 (t, $J=2.0$ Hz, 1H), 7.17 (t, $J=2.0$ Hz, 1H), 7.27 (t, $J=2.0$ Hz, 1H), 7.35 (d, $J=2.0$ Hz, 2H); Theoretical MS (M+H):554.1209; Found: 554.1197. Diastereomer **b**): ^1H NMR (400 MHZ, CD₃OD): δ (ppm) 2.47 (dd, $J=4.0, 16.0$ Hz, 1H), 2.56 (dd, $J=8.4, 16.0$ Hz, 1H), 3.30 (dd, $J=3.6, 12.4$ Hz, 2H), 3.44 (dd, $J=2.8, 12.4$ Hz, 2H), 3.94 (brs, 2H), 4.21-4.23 (m, 1H), 4.36-4.41 (m, 1H), 4.75 (d, $J=4.8$ Hz, 1H), 6.82 (t, $J=2.0$ Hz, 1H), 7.16 (t, $J=2.0$ Hz, 1H), 7.19 (t, $J=2.0$ Hz, 1H), 7.30 (t, $J=2.0$ Hz, 1H), 7.37 (d, $J=2.0$ Hz, 2H); Theoretical MS (M+H):554.1209; Found: 554.1164.

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EXAMPLE 20

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1**2**

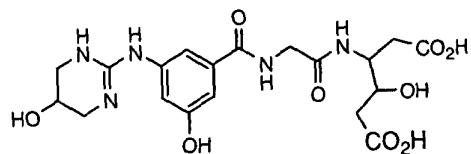
15

To a suspension of 5.3 g crude **2** and 4.6 g of compound **1** in 15 mL DMA at room temperature was added 608 mg HOBT followed by 2.58 mL N, N-diisopropylcarbodiimide. The resulting mixture was stirred at room temperature overnight to give a brown solution. To this solution was added 4.7 g LiOH·H₂O and 5 mL of water. Ethanol was added into the mixture till a clear solution was obtained. The progress of reaction was monitored by Mass spectrometry and analytical HPLC. After about 40 minutes, saponification completed. The basic solution was acidified to pH=1 with trifluoroacetic acid and purified by reverse phase preparative HPLC. ¹H NMR (300 MHZ, CD₃OD): δ (ppm) 2.54-2.62 (m, 1H), 2.74-2.81 (m, 1H), 3.12-3.54 (m, 4H), 4.02-4.10 (m, 2H), 4.38-4.31 (m, 2H), 4.65-4.74 (m, 1H), 6.85-6.9 (m, 1H), 7.19-7.23 (m, 2H); Theoretical MS (M+H):478.1550; Found: 478.1547.

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EXAMPLE 21

10 The title compound was prepared from the corresponding lactone according to the procedure described for the preparation of **EXAMPLE 10**.
15 ¹H NMR (400 MHZ, CD₃OD): δ (ppm) 2.34 (dd, *J*=9.2, 15.6 Hz, 1H), 2.54 (dd, *J*=8.4, 16.0 Hz, 1H), 2.62 (dd, *J*=3.2, 15.6 Hz, 1H), 2.73 (dd, *J*=4.0, 16.0 Hz, 1H), 3.31 (dd, *J*=3.6, 12.0 Hz, 2H), 3.44 (dd, *J*=2.8, 12.0 Hz, 2H), 3.94 (d, *J*=16.4 Hz, 1H), 4.01 (d, *J*=16.4 Hz, 1H), 4.01-4.07 (m, 1H), 4.18-4.23 (m, 2H), 6.82 (t, *J*=2.0 Hz, 1H), 7.17 (t, *J*=2.0 Hz, 1H), 7.19 (t, *J*=2.0 Hz, 1H); Theoretical MS (M+H): 468.1726; Found: 468.1731.

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5 Activity of the compounds of the present invention can be tested in
the following assays. Compounds of the present invention antagonize the
 $\alpha_v\beta_3$ integrin with an IC₅₀ of 0.1nM to 100 μ M in the 293-cell assay.
Similarly these compounds also antagonized the $\alpha_v\beta_5$ integrin with an IC₅₀
of < 50 μ M in the cell adhesion assay.

10

VITRONECTIN ADHESION ASSAY

Human vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are purified from human placenta as previously described [Pytela et al., Methods in Enzymology, 144:475-489 (1987)]. Human vitronectin is purified from fresh frozen plasma as previously described [Yatohgo et al., Cell Structure and Function, 13:281-292 (1988)]. Biotinylated human vitronectin is prepared by coupling NHS-biotin from Pierce Chemical Company (Rockford, IL) to purified vitronectin as previously described [Charo et al., J. Biol. Chem., 266(3):1415-1421 (1991)]. Assay buffer, OPD substrate tablets, and RIA grade BSA are obtained from Sigma (St. Louis, MO). Anti-biotin antibody is obtained from Sigma (St. Louis, MO). Nalge Nunc-Immuno microtiter plates were obtained from Nalge Company (Rochester, NY).

This assay is essentially the same as previously reported [Niiya et al., Blood, 70:475-483 (1987)]. The purified human vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are diluted from stock solutions to 1.0 μ g/mL in Tris-buffered saline containing 1.0 mM Ca⁺⁺, Mg⁺⁺, and Mn⁺⁺, pH 7.4 (TBS⁺⁺⁺). The diluted receptors are immediately transferred to Nalge Nunc-Immuno microtiter plates at 100 μ L/well (100 ng receptor/well). The plates are sealed and incubated overnight at 4°C to allow the receptors to bind to the wells. All remaining steps are at room temperature. The assay plates are emptied and 200 μ L of 1% RIA grade BSA in TBS⁺⁺⁺ (TBS⁺⁺⁺/BSA) are added to block exposed plastic surfaces. Following a 2 hour incubation, the assay plates are washed with TBS⁺⁺⁺ using a 96 well plate washer. Logarithmic serial dilution of the test compound and controls are made starting at a stock concentration of 2 mM and using 2 nM biotinylated vitronectin in TBS⁺⁺⁺/BSA as the diluent. This premixing of labeled ligand

5 with test (or control) ligand, and subsequent transfer of 50 µL aliquots to the assay plate is carried out with a CETUS Propette robot; the final concentration of the labeled ligand is 1 nM and the highest concentration of test compound is 1.0×10^{-4} M. The competition occurred for two hours after which all wells are washed with a plate washer as before. Affinity

10 purified horseradish peroxidase labeled goat anti-biotin antibody is diluted 1:2000 in TBS⁺⁺⁺/BSA and 125 µL is added to each well. After 45 minutes, the plates are washed and incubated with OPD/H₂O₂ substrate in 100 mM/L Citrate buffer, pH 5.0. The plate is read with a microtiter plate reader at a wavelength of 450 nm and when the maximum-binding control wells

15 reached an absorbance of about 1.0, the final A₄₅₀ are recorded for analysis. The data are analyzed using a macro written for use with the EXCEL spreadsheet program. The mean, standard deviation, and %CV were determined for duplicate concentrations. The mean A₄₅₀ values are normalized to the mean of four maximum-binding controls (no competitor

20 added)(B-MAX). The normalized values are subjected to a four parameter curve fit algorithm [Rodbard et al., Int. Atomic Energy Agency, Vienna, pp 469 (1977)], plotted on a semi-log scale, and the computed concentration corresponding to inhibition of 50% of the maximum binding of biotinylated vitronectin (IC₅₀) and corresponding R² is reported for those

25 compounds exhibiting greater than 50% inhibition at the highest concentration tested; otherwise the IC₅₀ is reported as being greater than the highest concentration tested.

PURIFIED IIb/IIIa RECEPTOR ASSAY

30 Human fibrinogen receptor ($\alpha_v\beta_3$) is purified from outdated platelets. (Pytela, R., Pierschbacher, M.D., Argraves, S., Suzuki, S., and Rouslahti, E. "Arginine-Glycine-Aspartic acid adhesion receptors", Methods in Enzymology 144(1987):475-489.) Human vitronectin is purified from fresh frozen plasma as described in Yatohgo, T., Izumi, M., Kashiwagi, H., and

35 Hayashi, M., "Novel purification of vitronectin from human plasma by heparin affinity chromatography," Cell Structure and Function 13(1988):281-292. Biotinylated human vitronectin is prepared by coupling

5 NHS-biotin from Pierce Chemical Company (Rockford, IL) to purified
vitronectin as previously described. (Charo, I.F., Nannizzi, L., Phillips, D.R.,
Hsu, M.A., Scarborough, R.M., "Inhibition of fibrinogen binding to GP IIb/IIIa
by a GP IIIa peptide", J. Biol. Chem. 266(3)(1991): 1415-1421.) Assay
buffer, OPD substrate tablets, and RIA grade BSA are obtained from Sigma
10 (St. Louis, MO). Anti-biotin antibody is obtained from Sigma (St. Louis,
MO). Nalge Nunc-Immuno microtiter plates are obtained from (Rochester,
NY). ADP reagent is obtained from Sigma (St. Louis, MO).

This assay is essentially the same reported in Niiya, K., Hodson, E.,
Bader, R., Byers-Ward, V. Koziol, J.A., Plow, E.F. and Ruggeri, Z.M.,
15 "Increased surface expression of the membrane glycoprotein IIb/IIIa
complex induced by platelet activation: Relationships to the binding of
fibrinogen and platelet aggregation", Blood 70(1987):475-483. The purified
human fibrinogen receptor ($\alpha_v\beta_3$) is diluted from stock solutions to 1.0
 μ g/mL in Tris-buffered saline containing 1.0 mM Ca⁺⁺, Mg⁺⁺, and Mn⁺⁺, pH
20 7.4 (TBS⁺⁺⁺). The diluted receptor is immediately transferred to Nalge
Nunc-Immuno microtiter plates at 100 μ L/well (100 ng receptor/well). The
plates are sealed and incubated overnight at 4°C to allow the receptors to
bind to the wells. All remaining steps are at room temperature. The assay
plates are emptied and 200 μ L of 1% RIA grade BSA in TBS⁺⁺⁺
25 (TBS⁺⁺⁺/BSA) are added to block exposed plastic surfaces. Following a 2
hour incubation, the assay plates are washed with TBS⁺⁺⁺ using a 96 well
plate washer. Logarithmic serial dilution of the test compound and controls
are made starting at a stock concentration of 2 mM and using 2 nM
biotinylated vitronectin in TBS⁺⁺⁺/BSA as the diluent. This premixing of
30 labeled ligand with test (or control) ligand, and subsequent transfer of 50
 μ L aliquots to the assay plate is carried out with a CETUS Propette robot;
the final concentration of the labeled ligand is 1 nM and the highest
concentration of test compound is 1.0×10^{-4} M. The competition occurred
for two hours after which all wells are washed with a plate washer as
35 before. Affinity purified horseradish peroxidase labeled goat anti-biotin
antibody is diluted 1:2000 in TBS⁺⁺⁺/BSA and 125 μ L are added to each

5 well. After 45 minutes, the plates are washed and incubated with
ODD/H₂O₂ substrate in 100 mM/L citrate buffer, pH 5.0. The plate was
read with a microtiter plate reader at a wavelength of 450 nm and when the
maximum-binding control wells reached an absorbance of about 1.0, the
final A₄₅₀ are recorded for analysis. The data are analyzed using a macro
10 written for use with the EXCELJ spreadsheet program. The mean,
standard deviation, and %CV are determined for duplicate concentrations.
The mean A₄₅₀ values are normalized to the mean of four maximum-
binding controls (no competitor added)(B-MAX). The normalized values
are subjected to a four parameter curve fit algorithm, [Robard et al., Int.
15 Atomic Energy Agency, Vienna, pp 469 (1977)], plotted on a semi-log
scale, and the computed concentration corresponding to inhibition of 50%
of the maximum binding of biotinylated vitronectin (IC₅₀) and corresponding
R² was reported for those compounds exhibiting greater than 50% inhibition
at the highest concentration tested; otherwise the IC₅₀ is reported as being
20 greater than the highest concentration tested. β-[[2-[[5-
[(aminoiminomethyl)amino]-1-oxopentyl]amino]-1-oxoethyl]amino]-3-
pyridinepropanoic acid [US 5,602,155 Example 1] which is a potent α_vβ₃
antagonist (IC₅₀ in the range 3-10 nM) is included on each plate as a
positive control.

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Human Platelet Rich Plasma Assays

Healthy aspirin free donors are selected from a pool of volunteers.
The harvesting of platelet rich plasma and subsequent ADP induced
platelet aggregation assays are performed as described in Zucker, M.B.,
30 "Platelet Aggregation Measured by the Photometric Method", Methods in
Enzymology 169(1989):117-133. Standard venipuncture techniques using
a butterfly allowed the withdrawal of 45 mL of whole blood into a 60 mL
syringe containing 5 mL of 3.8% trisodium citrate. Following thorough
mixing in the syringe, the anti-coagulated whole blood is transferred to a 50
35 mL conical polyethylene tube. The blood is centrifuged at room
temperature for 12 minutes at 200 xg to sediment non-platelet cells.
Platelet rich plasma is removed to a polyethylene tube and stored at room

5 temperature until used. Platelet poor plasma is obtained from a second centrifugation of the remaining blood at 2000 xg for 15 minutes. Platelet counts are typically 300,000 to 500,000 per microliter. Platelet rich plasma (0.45 mL) is aliquoted into siliconized cuvettes and stirred (1100 rpm) at 37°C for 1 minute prior to adding 50 uL of pre-diluted test compound. After
10 1 minute of mixing, aggregation is initiated by the addition of 50 uL of 200 uM ADP. Aggregation is recorded for 3 minutes in a Payton dual channel aggregometer (Payton Scientific, Buffalo, NY). The percent inhibition of maximal response (saline control) for a series of test compound dilutions is used to determine a dose response curve. All compounds are tested in
15 duplicate and the concentration of half-maximal inhibition (IC_{50}) is calculated graphically from the dose response curve for those compounds which exhibited 50% or greater inhibition at the highest concentration tested; otherwise, the IC_{50} is reported as being greater than the highest concentration tested.

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Cell Assays for Potency and Selectivity

While the β_3 subunit of $\alpha_v\beta_3$ is only known to complex with α_v or α_{IIb} , the α_v subunit complexes with multiple β subunits. The three α_v integrins most homologous with $\alpha_v\beta_3$ are $\alpha_v\beta_1$, $\alpha_v\beta_5$ and $\alpha_v\beta_6$, with 43%, 56% and 47
25 % amino acid identity in the β subunits, respectively. To evaluate the selectivity of compounds between the integrins $\alpha_v\beta_3$ and $\alpha_v\beta_6$, cell-based assays were established using the 293 human embryonic kidney cell line. 293 cells express $\alpha_v\beta_1$, but little to no detectable $\alpha_v\beta_3$ or $\alpha_v\beta_6$. cDNAs for β_3 and β_6 were transfected separately into 293 cells to generate 293- β_3 and
30 293- β_6 cells, respectively. High surface expression of $\alpha_v\beta_3$ and $\alpha_v\beta_6$ was confirmed by flow cytometry. Conditions were established for each cell line in which cell adhesion to immobilized human vitronectin was mediated by the appropriate integrin, as determined by a panel of integrin-specific, neutralizing monoclonal antibodies. Briefly, cells were incubated with
35 inhibitor in the presence of 200uM Mn²⁺, allowed to adhere to immobilized vitronectin, washed, and adherent cells are detected endogenous alkaline

5 phosphatase and para-nitrophenyl phosphate. An 8-point dose-response curve using either 10-fold or 3-fold dilutions of compound was evaluated by fitting a four-parameter logistic, nonlinear model (using SAS).
To evaluate compound potency for membrane-bound $\alpha_v\beta_6$ an additional cell-based adhesion assay was established using the HT-29 human colon
10 carcinoma cell line. High surface expression of $\alpha_v\beta_6$ on HT-29 cells was confirmed by flow cytometry. Conditions were established in which cell adhesion to immobilized human latency associated peptide (LAP) was mediated by the $\alpha_v\beta_6$, as determined by a panel of integrin-specific, neutralizing monoclonal antibodies. Briefly, cells were incubated with
15 inhibitor in the presence of 200uM Mn²⁺, allowed to adhere to immobilized LAP, washed, and adherent cells are detected by quantifying endogenous alkaline phosphatase using para-nitrophenyl phosphate. An 8-point dose-response curve using either 10-fold or 3-fold dilutions of compound was evaluated by fitting a four-parameter logistic, nonlinear model (using SAS).
20 The compounds evaluated were relatively ineffective at inhibition of $\alpha_v\beta_6$ -mediated cell adhesion. The selective antagonism of the $\alpha_v\beta_3$ integrin is viewed as desirable in this class of compounds, as $\alpha_v\beta_6$ may also play a role in normal physiological processes of tissue repair and cellular turnover that routinely occur in the skin and pulmonary tissues.

25